Ph.D. Thesis

In vitro and in vivo evaluation of semisolid dosage forms for transdermal application of Ketamine

George Zapantis
Pharmacist

Szeged
Hungary
2007

Ph.D. Thesis

University of Szeged
(Albert Szent-Györgyi Medical University)
Faculty of Pharmacy
Department of Pharmaceutical Technology
Director: Prof. Dr. habil. Piroska Szabó-Révész, Ph.D., D.Sc.

In vitro and in vivo evaluation of semisolid dosage forms for transdermal application of Ketamine

By
George Zapantis
Pharmacist

Supervisors:
Prof. Dr. habil. Istvan Erős, Ph.D., D.Sc.
and
Dr. habil. Ildikó Csóka Ph.D

Szeged
Hungary
2007

TABEL OF CONTENTS

1. INTRRODUCTION

In the past decade there has been an increase interest toward dermal and transdermal products which offer several advantages compared to traditional dosage forms [1-3]. USA data show that out of 129 drug delivery candidates, 51 dermal or transdermal products are listed. There are 77 candidate products in preclinical development of which 30% represent such drug delivery [4]. Novel approaches and devices have been manufactured in recent years [2, 5]; parallelly with the development of in vitro and in vivo test methodologies [6-10] and mathematical description of the processes involved in skin delivery [11, 12].

These new innovations (dosage forms, devices) made several active agents useful therapeutically, which couldn't serve as medicinal cures before, due to their sensitive structures (e.g. peptides), or because a lack of suitable devices or methodologies to carry them through e.g. the skin layers.

Paralelly with these new possibilities, there is also an increasing demand from clinicians' side toward non-invasive therapies - like transdermal delivery - generally and also in the case of special patient groups, such as children.

These abovementioned facts give drive to dosage form designers for creating novelties and making efforts do develop new dosage forms and widen their usage to incorporate more medicinal substances.

This work was initiated by the clinicians' side, as there was a need for a non-invasive technological delivery alternative of Ketamine for pediatric usage.

2. AIM

Clinicians for a while have been looking for an alternative for the induction of anesthesia. This procedure has been normally preformed by inhalation, injection, or intubation, while being uncomfortable for adults; is painful for children, and can have long-term psychological side effects. A technological alternative is very much in demand. I have chosen Ketamine, a dissociative anesthesia, for the induction of anesthesia by the transdermal route. Ketamine hydrochloride is a water-soluble salt, which has difficulty in penetration of the skin to be able to have systemic effect.

My project was to take one representative of each of the following 4 different vehicles: hydrogel, organogel, liquid crystals and emulsions (w\o, w\o\w) to use for the application of Ketamine hydrochloride. The result will be to examine which vehicle will provide the best penetration for the drug to be able to formulate the right design for transdermal application in the form of a transdermal patch as an end result.

The process will include:

1. In vitro experiments with the Hanson Vertical Diffusion Cell Apparatus with
    a) Phosphate soaked membrane,
    b) IPM soaked membrane.

This is to examine if the drug can penetrate the artificial membrane and to determine the feasibility of proceeding to in vivo experimentation.

2. In vivo experiments with the application of the vehicle on the skin of Wistar rats

This is to observe the actual amount of drug penetration and the effect the drug has on the physiological functions.

3. Blood will be taken from the Wistar rats to measure plasma levels of Ketamine, with an HPLC apparatus to assest the actual penetration effect each vehicle has on the skin.

3. LITERATURY SURVEY

3.1 General overview of transdermal drug delivery

There have been large studies in topical delivery research during the past 60 years. The years between 1940 and 1980 have been formative years for the current understanding of transdermal delivery. Part of the difficulties was in accessing the information from the databases. In the 50's and the 60's there was research to identify the barrier properties of the skin, in the 60's Higuchi introduced mathematical modeling of percutaneous penetration, in the late 60's thermodynamic activity (or chemical potential) was studied in terms of supersaturation, and in the 70's when the structure of the Stratum Corneum (SC) was found to be like a brick and mortar wall they understood that intracellular route was the transport route for the drug in the SC and it is this structure that gives the skin a rate controlling membrane. Finally it was in the mid 70' that Franz invented the Franz diffusion cell for in vitro analysis and during this time various membranes started being used for testing like cellulose acetate [13].

The concept of a transdermal patch was introduced in the 1970's [14]. The first transdermal patch manufactured was used for systemic effect with the active ingredient scopolamine to treat motion sickness. It was made in 1981 by Ciba-Geigy using the name Transderm V (It is now sold as Transderm Scop) [15]. Walters and Roberts [14] defined the basic interests in the dermal absorption of drugs and compounds, such as: local effect (corticosteroids), systemic effect (nicotine patch), surface effect (sunscreens, cosmetics), and deeper effect (NSAID's agents for muscle inflammation), which gave the basis also for patch designers to develop this possibility for various drugs that now are available on the market in the form of a transdermal patch. They include: Nitroglycerine (angina) [16], Scopolamine (motion sickness) [17], Fentanyl (pain) [18], Nicotine (smoking cessation) [19], Estrogen (hormone replacement therapy) [20, 21], Testosterone (male hypogonadism) [22], Clonadine (hypertension) [23], Lidocaine (local anesthesia) [24].

There are also new transdermal patches that are now available or will be available soon on the market. They include medicated: Lidocaine (pain of shingles in Herpes Zoster), Methylphenidate (attention deficit hyperactivity disorder ADHD) [25, 26] and non-medicated: thermal and cold patches, weight loss patch, nutrient patch, therapeutic and cosmetic patches, aroma patch for appetite suppression, and a patch to measure sunlight exposure [27].

3.1.1 Advantages and disadvantages of transdermal application of drugs

Advantages of transdermal application of drugs are as follows [14, 15, 28-30]:

1. Avoid stomach emptying (pulsed erratic delivery of drugs to the intestine), pH effects (acid can degrade the drug in the stomach), GI damage (drugs like NSAID's can cause micro bleeding in the stomach), and enzyme deactivation in the GI passage.
2. No hepatic first pass metabolism.
3. Control of initiation and termination.
4. Patient compliance is increased.
5. Controlled release of a drug that has a narrow therapeutic index.
6. Less dosing is needed for a medication with a short half-life (<5 Hours).
7. Drugs with high toxicity can be used.
8. Avoids the "Peaks and Valleys" of drug plasma levels found in other administration routes.

Disadvantages of transdermal application of drugs are as follows [14, 15, 28, 31]:

1. Variation of absorption due to place of application, disease, age, species, races.
2. Reservoir capacity of the skin.
3. Metabolic effects of the skin (bacteria and enzymes).
4. Toxicity and irritation of the skin.
5. Few candidates available for administration.
6. A patient wearing a transdermal patch does not inform the doctor in the hospital and an incompatibility or an overdose can occur.
7. Some transdermal patches contain metal and have to be removed before an MRI exam if not then burning of the underlying tissue can occur.

3.1.2 Types of semisolid drug delivery systems and transdermal patches

Selection of a suitable vehicle is very important in topical and transdermal formulations, as it can affect both drug release and percutaneous absorption. Factors that

contribute to the selection of a suitable vehicle are: (a) solubility of the drug in the vehicle; (b) release of the drug from the vehicle into the skin; and (c) enhancement of drug penetration through the SC. In a pharmaceutically acceptable product, the ideal drug vehicle is compatible and complementary, able to promote drug absorption and to alleviate the skin disorder by 'cosmetic' means. Increased skin hydration has been shown to result in increased drug absorption from topical formulations. For example, occlusion, a well-known technique, enhances the absorption of many drugs, including both hydrophobic and hydrophilic compounds, by increasing skin hydration. Surfactants have traditionally been used in topical pharmaceutical preparations, including hydrophilic ointments, absorption bases and emulsion-type creams. Their usual role is to aid in drug solubilization and to attribute water washability to the vehicle for cosmetic appeal. Through interactions with the skin, delivery vehicles provide a complementary action by which the skin can be 'prepared' to allow passage of the drug. Because active agents can have widely different solubility, sizes and structures, different agents would require different 'preparation' of the skin by appropriate delivery vehicles [1].

Emulsions and creams are very popular among the dosage forms used in topical delivery, where the main components are lipids and water; stabilized by emulsifiers. Developments within this field are the usage of vegetable oils and new types of surfactants e.g. POE free, and also new emulsion types are used as well. One of the most popular new emulsion types is the water-in-oil-in-water (W/O/W) multiple emulsions; where small water droplets are entrapped within larger oil droplets that in turn are dispersed in a continuous water phase. Multiple W/O/W emulsions contain both W/O and O/W simple emulsions and requires at least 2 emulsifiers to be present in the system [32-34].

Lyotropic liquid crystals are fairly new members of topical dosage forms, formed by amphiphilic molecules and exhibit a phase of matter that has properties between those of a conventional liquid, and those of a solid crystal [35-37].

In contrast to emulsions, gels generally do not comprise two immiscible phases of opposite lipophilicity. Therefore, the polarity and solubility characteristics of the incorporated substances are either hydrophilic in hydrogel or lipophilic in organogel. The consistency of gels is caused by gelating agents, who belong mainly to polymers (but they can be emulsifiers as well). These polymers build up a three dimensional network. Intermolecular forces bind the solvent molecules to this polymeric network and thus, due to the reduced mobility of these molecules in structured systems with increased viscosity, exhibit viscoelastic properties. The most important and already well-known polymers for

forming hydrogels are polyacrylic acid derivatives like carbomers, different cellulose derivatives like hydroxyethyl cellulose, hydroxypropyl cellulose and croscarmellose-sodium etc. [38-39].

In case of organogel, sorbitan monostearate, a hydrophobic nonionic surfactant, gelifies a number of organic solvents such as hexadecane, isopropyl myristate, and a range of vegetable oils. Gelation is achieved by dissolving/dispersing the organogelator in hot solvent to produce an organic solution/dispersion, which on cooling sets to the gel state. Cooling the solution/dispersion causes a decrease in the solvent-gelator affinities, such that at the gelation temperature, the surfactant molecules self-assemble into toroidal inverse vesicles. Further cooling results in the conversion of the toroids into rod-shaped tubules. Once formed, the tubules associate with others, and a three-dimensional network is formed which immobilizes the solvent. An organogel is thus formed. Sorbitan monostearate gels are opaque, thermo reversible semisolids, and they are stable at room temperature for weeks. The gels are affected by the presence of additives such as the hydrophilic surfactant, Polysorbate 20, which improves gel stability and alters the gel microstructure from a network of individual tubules to star-shaped "clusters" of tubules in the liquid continuous phase. Another solid monoester in the sorbitan ester family, sorbitan monopalmitate, also gels organic solvents to give opaque, thermo reversible semisolids. Like sorbitan monostearate gels, the microstructure of the palmitate gels comprises an interconnected network of rodlike tubules. Unlike the stearate gels, however, the addition of small amounts of a polysorbate monoester causes a large increase in tubular length instead of the "clustering effect" seen in stearate gels. The sorbitan stearate and palmitate organogel may have potential applications as delivery vehicles for drugs and antigens [40].

These above mentioned dosage forms can be used in their semisolid form, but also their incorporation into transdermal patches is a common process. In this case these patches have their own device specific parts, as follows: the basic design of a transdermal patch is a release liner, pressure-sensitive adhesive, and a backing layer. There are 3 basic transdermal delivery systems that can be described by their basic design [27, 29, 41].

1. Drug in adhesive – The drug is incorporated into the adhesive
2. Drug in matrix – The drug is dispersed in a polymeric matrix
3. Drug in reservoir (membrane patch) – There is a rate-controlling membrane between the reservoir and the skin.

This work does not detail these devices, as the basic aim was the development and testing of the dosage form itself.

3.2 Theoretical background of skin absorption

3.2.1 Skin factors relevant to transdermal absorption of active agents

The skin being the largest organ of the body covers an area of $2m^2$ and is on the average 0.5 mm thick. The skin as a barrier can withstand various degrees of temperature and water content [1, 14, 42-45]. The skin is composed of three layers [1, 14, 46] and several appendages [1, 14], which contribute to the homeostasis function of the skin [47]. According to the different skin layers each has unique characteristics witch influence the penetration of the drug into the body.

The stratum corneum (SC) is the outermost membrane of the skin that consists of a heterogeneous membrane with lipids and proteins in a "Brick and Mortar" morphology giving it its barrier function [1, 13, 14, 29, 42]. The lipid concentration will give the SC a lipophilic environment and it is through this barrier that the drug has to partition first. The first layer is the epidermal layer. It senses damage caused by substances that pass the SC and either metabolizes it or causes an inflammatory response [1, 14, 48]. This layer has an aqueous environment and acts as a hydrophilic layer of which lipophilic drugs have difficulty partitioning into. The following layer is the dermal layer, which supplies nutritive and immune support to the epidermis. This is due to the neural and blood supply of the dermis [1, 14]. When the drug reaches this layer it is distributed to the body by the blood supply for elimination or systemic effect [46]. Finally the last layer is the subcutaneous tissue and is composed of a network of fat cells, which carry the vascular and neural systems for the skin [14]. Depending on the state of the vascular supply it will determine the target of the drug. In a vasodilated state the drug will remain local and in a vasoconstricted state the drug will be transported to the systemic circulation.

According to Sarber and Davis [49] there are various factors that influence the absorption of a drug from topical preparations which include: skin microflora, skin pH, cutaneous blood flow, surface lipids, anatomical site of application, and influence of appendages. There are also other factors that have been studied which are: diseased or

damaged skin [51], skin metabolism [28, 50], age of skin [50, 51], variation of races [51], variation of species [28, 50], and reservoir capacity of the skin [28].
All these facts are to be taken into consideration when formulating such products.

3.2.2 Theoretical background of diffusion through the skin layers

Percutaneous absorption of pharmaceuticals for local or systemic delivery can be obtained by the introduction of the substance and other compounds to the skin with the appropriate dosage forms [28]. The movement of the drug across the SC is a passive process that follows Fick's law of diffusion [44, 46, 52-54], described in Equation 1.

$$J = -D*A\,{}^{\partial c}/_{\partial x} \quad (1)$$

Where

J = the steady state flux of the drug,

D = the diffusion coefficient of the drug into the SC,

A = the surface area of the membrane,

${}^{\partial c}/_{\partial x}$ = the concentration gradient or the driving force across the SC.

This diffusion process can happen in three major routes: intercellular (paracellular) [28, 46, 50, 54, 55], which is the most preferred route of penetration for the drug through the SC; transcellular (transcytosis) [28, 46, 50, 54, 56], which is thought to be the main route for penetration but due to the fact there is repeated partitioning of the drug between the lipophilic and hydrophilic compartments this pathway makes it difficult for transport. The transappendageal (eccrine glands or hair follicles) [28, 44, 57, 58] pathway dominates the transport of the drug in the lag time, but during longer durations transcellular dominated, but it is accepted that during steady-state penetration the intracellular route is dominant. This can be an alternative pathway for drug transport.

The advantages in being able to make accurate predictions of the extent to which substances penetrate through human skin are very evident in estimating the potential for transdermal drug delivery, in assessing the risk associated with dermal contacts with toxic substances and in the cosmetics industry. Modelling such dermal exposures has as its ultimate objective the development of expert systems capable of reliably predicting the

extent to which a molecule will be percutaneously absorbed, without the need to make experimental measurements. Because of the relative complexity of human skin and of many of the penetrant molecules of interest it is not yet possible to calculate the relevant diffusion barriers from first principles as has now been done for simpler systems [59, 60]. Neither is it possible to use simulation methods, such as molecular dynamics, that rely on interatomic potentials to represent the molecular forces acting between the species in the system. Two principal approaches have emerged. The first is the use of quantitative structure–activity relationships (QSARs) that are used mainly to predict steady-state permeabilities. The second utilises mathematical models to simulate the sequence of partition and transport processes involved in the absorption and can predict the extent and rate of chemical permeation through the skin. QSARs are widely used in science to statistically correlate selected relevant physicochemical properties of compounds with their biological activities [61]. Quite a number have now been developed specifically to model skin permeation and this field has been the subject of comprehensive reviews [62, 63]. These algorithms typically predict the permeability (K_p) of the substance through the SC, as it is described described in Equation 2.

$$K_p = K_m \times D_m/h \quad (2)$$

Where

h = the thickness of the SC,

D_m = the permeant diffusivity in the membrane,

K_m = partition coefficient between the SC and the vehicle.

In calculations, K_m is often substituted by the octanol–water partition coefficient (K_{ow}). The general QSAR based on the Flynn [64] dataset and reported by Potts and Guy [65] established the use of a combination of the octanol-water partition coefficient, K_{ow}, and the MW or molecular volume as physicochemical descriptors as being both mechanistically relevant and capable of providing an adequate interpretation of these data. This QSAR has the form of Equation 3 [66]:

$$\text{Log}K_p = 0{:}71 \log K_{ow} - 0{,}0061MW - 6{,}3 \quad (3)$$

3.3 Development of transdermal drug delivery systems

According to Kalia et.al. [67] and Morganti et. al. [68], the path that the drug follows is (a) release of the drug from the formulation, (b) partition into the SC, (c) diffusion through the lipophilic environment of the SC, (d) partition into the aqueous environment of the epidermis, (e) diffusion through the epidermis to the dermis, and finally (f) taken up by the capillary network to the systemic circulation. When examining the route the drug has to take to penetrate the skin we have to take into consideration the polarity of the SC (lipophilic) and the epidermis (hydrophilic) when choosing a drug as a candidate for transdermal delivery. The drug must possess both lipoidal and aqueous solubility. A drug that is too hydrophilic will not transfer into the SC and if it is too lipophilic it will remain in the SC [69].

Transdermal drug delivery can be optimized by various methods. These methods vary from changing the drug parameters, choosing the proper vehicle and/or application of special additives called enhancers (chemical and physical) in order to modify the barrier function of the skin.

3.3.1 Modification of the drug parameters

To be a candidate for transdermal delivery a drug must have certain characteristics, which are: a molecular weight of 500 Dalton (<300 Dalton is preferred), melting point should be 200° C (>150° C is preferred), the polarity should not be very hydrophilic or very lipophilic. There should be no skin toxicity of irritancy, the dosage should be up to 50mg daily, and the therapeutic blood concentration should be in the ng ml^{-1} range or less [69-71]. These requirement are difficult to fulfill, therefore there are several possibilities available to achieve this goal, as follow:

1. Drug or prodrug – A drug must have certain requirements to be able to be absorbed. If a drug does not fit these requirements a prodrug can be used. After permeation the enzymes in the skin activate the drug [1, 42, 53]. Ketorolac amide prodrug [72], Temozolomide ester prodrug [73] are currently being used.
2. Adjustment of chemical potential – Use of a supersaturated solution either by design or by cosolvent evaporation on the skin increases the chemical potential of the drug there by increasing the driving force [42, 44, 53, 54, 57, 67, 74, 77].
3. Use of ion pairs – Since charged molecules cannot readily penetrate the SC a lipophilic ion pair (two opposite charged species together) is used. The ion pair after

diffusion to the aqueous viable epidermis dissociates into its charged species and diffuses onwards [42].

3.3.2 The role of vehicles in drug delivery and absorption

Another possibility for alteration of drug absorption through the skin layers is to develop special dosage forms beside the traditionally used ones, such as:

1. Liposomes, which are spherical vesicles with a membrane composed of a phospholipid bilayer that entraps the drug and delivers it to the skin, which it then fuses with the SC lipids and the structure of the vehicle collapses liberating the drug [1, 4, 42, 43, 45, 57, 76-78].
2. Transferosomes are similar to liposomes in that they have a lipid bilayer that surrounds an aqueous core. When a suspension of a transferosome vesicle is placed on the surface of the skin the water evaporates from the vesicles and starts to dry out. Due to the strong hydrophilicity of major transferosome ingredients the vesicles are attracted to the areas of higher water content in the narrow gaps between adjoining cells in the skin. They temporarily open the pores, which create channels between the skin cells allowing penetration [4, 42, 43, 45, 57, 78].
3. Ethosomes contain soft phospholipid vesicles in the presence of high concentrations of ethanol, which fluidizes the lipid bilayer [4, 42, 43, 45, 79].
4. Niosomes have a similar structure to liposomes but have no phospholipids and instead contain nonionic surfactants [4, 42, 43, 45, 57, 78].
5. High velocity particles – Solid particles are fired through the SC into the lower skin layers. This PowderJect system uses a supersonic shock wave of helium gas [42].

3.3.3 Modification of the barrier function by chemical enhancers

The penetration of the drug into the skin can be increased with the use of enhancers by either disruption of the lipids barrier or by increasing partitioning promotion or a combination of both. The enhancers enter the lipid bilayer forming micro cavities or channels thereby increasing the free volume available for drug diffusion or they open up the dense structure of the SC increasing permeability, examples are; urea, water, DMSO, Azone, fatty acids (Oleic Acid), Terpenes. The second type behavior is, when the enhancer

alters the solubility properties of the tissue thereby increasing the drug partitioning into the membrane, examples are: Pyrrolidones, alcohols (ethanol), glycols (Propylene Glycol). A combination of both actions happens, when the enhancer not only disrupts the bilayer but also alter the solubility of it, examples are: surfactants (ionic, cationic, and non-ionic). According to Asbill [81] the enhancer's effects can be put into the following groups:

1. The diffusion of the drug into the skin is increased.
2. A reversible decrease of the barrier function in the SC by lipid-fluidization.
3. The drugs thermodynamic activity in the skin and the vehicle is increased.
4. A reservoir of the drug is formed in the skin.
5. The partition cofficient of the drug is affected causing an increase of the release from the formulation into the upper layers of the skin.

Barry [4] has listed the properties of penetration enhancers as:

1. Pharmacologically inert – Does not cause pharmacological activity.
2. Work is unidirectional – It allows substances to enter and inhibit loss of endogenous material.
3. Activity is immediate – The duration and the activity of the enhancer can be predicted.
4. Reversibility – The barrier properties of the skin should return immediately and completely when the enhancer is removed.
5. Compatibility – There should be compatibility with the recipients, the drug, and the enhancer.
6. Does not cause damage to the skin – The enhancer should be non-toxic, non-irritating, and non-allergenic.
7. Acceptance to the patient – There should be an acceptable feeling to the patient during the application.
8. Physical characteristics – Odorless, colorless, and tasteless.

No material to this date has been able to possess all the mentioned properties.

Williams and Barry [76] stated that concerning penetration enhancers:

1. The selection of a penetration enhancer is difficult due to the fact that their potencies seem to be drug specific.
2. Animal and rodent skins show greater penetration enhancement than human skin.
3. There is a better result when the penetration enhancer is used with a co-solvents.

4. The majority of the penetration enhancers are concentration dependent.
5. Penetration enhancers can act directly on the skin by decreasing the barrier properties of the SC, or by modifing the solvent nature of the SC. They can also act indirectly by modifying the thermodynamic activity of the vehicle (ex. supersaturation), by making the permeant soluble in the donor (ex. surfactants), or permeate the skin and take the drug with it in what is called "solvent drag".

Some examples for penetration enhancing substances include:

Water – Hydration of the SC opens the compact structure and increases penetration rate. Water forms pools in the SC that connect to form a pathway by which the drug can penetrate [1, 28, 45, 76, 80, 84].

Urea – It is a hydrating agent (a hydrotrope) that is used in treatment of hyper-keratotic skin conditions (psoriasis, ichthyosis). The possible mechanism is by increasing the water content of the SC and by keratolytic activity. Urea is a moderate penetration enhancer [76, 82-85].

DMSO – It is an aprotic (universal) solvent and one of the earliest penetration enhancers. It is concentration dependent and >60% is needed for optimum enhancement but at this concentration causes skin irritation. The mechanism is disruption of the lipid bilayer [1, 76, 82, 84, 86].

Azone – It is the first molecule formed as a penetration enhancer and it has fewer disadvantages than DMSO. Azone distributes itself homogenously in the lipid domains of the SC and forms pools within the bilayers. Like DMSO Azone is concentration dependent except only low concentrations is needed. The mechanism is the disruption of the bilayer lipids and their packing arrangement. [1, 53, 57, 75, 76, 82, 84].

*Fatty Acid*s – The most commonly used fatty acid is oleic acid. If the fatty acid is saturated then the optimal length for penetration is around C_{10}-C_{12}. If it is unsaturated the optimal length is C_{18}. It is effective at low concentrations (less than 10%). The mechanism is the modification of the lipid domains of the SC and at high concentrations forms pools within the lipid baitlayers [1, 53, 75, 76].

Terpenes – They are compounds that are found in essential oils and are well-established penetration enhancers but some terpens have pharmacological activity. Smaller terpins are more active than the larger sequiterpenes. The mechanism is the disruption of the intercellular lipid barrier [76, 79, 82, 87].

Pyrrolidones – They penetrate the SC where they alter the solvent nature of the membrane and have also shown to generate "reservoirs" within the skin membranes, which may have the potential for sustained release of a permeate. Pyrrolidones may cause skin irritation [76, 82, 84].

Alcohols (ethanol) – Ethanol is the solvent of choice for use in transdermal patches. The mechanism is the increase of the solubility of the drug in the vehicle and permeation of ethanol into the SC, which alters the solubility properties of the tissue thereby increasing the partitioning of the drug into the membrane [1, 76, 82, 84, 88].

Glycols – Propylene Glycol is the most commonly used glycol with concentrations of 1-10% the mechanism is similar to ethanol. A low concentration (1-10%) is needed for effect. The mechanism is altering the solubility characteristics of the skin [1, 53, 76, 82, 84, 89].

Surfactants – The most common use of surfactants is to solubilize lipophilic active ingredients and to solubilize the lipids within the SC. Their main mechanism is the ability to be absorbed at interfaces which leads to a reduction of the interfacial tension. The action of the surfactants is concentration-dependent where the enhancement of membrane transport occurs at low concentrations of the surfactant below the critical micelle concentration (cmc). The mechanism of membrane transport at low surfactant concentrations is the penetration and the disruption of the cell membrane structure [1, 76, 82, 84, 90].

3.3.4 Mechanical methods for penetration of the SC

One possibility is to create holes in the SC mechanically. This is achieved by needles or knives attached to a skin patch. The needles can be in a microneedle array where 400 hollow microneedles penetrate the SC but do not reach the nerve endings so there is no pain sensation. This process will open pores in the SC allowing the drug to penetrate [91-95].

Chemical peels, microdermabrasion, laser ablation, and adhesive tape can remove the horny layer that usually provides a permeation barrier. When the horny layer of the skin is removed there is a thousand times more rapid diffusion of water-soluble non-electrolytes into the systemic circulation [42].

Ultrasound (phonophoresis, sonophoresis) – energy causes cavities (tiny holes are created) in the SC disturbing the lipid packing and as a result increases the free volume space where the drugs can pass [42, 53, 96-100].

Iontophoresis (low intensity electric current) is the electrical driving of charged species into tissue. Wires to skin-interfacing electrodes connect a device that discharges an electrical impulse. The mechanisms are: the electrical repulsion of the charged species from the driving electrode, the electrical current can increase permeability of the skin, and/or electroosmosis [4, 42, 45, 53, 101-103]

Electroporation (electropermeabilization) is where a brief controlled electric pulsed field is applied to living cells. It uses short electrical pulses forming transient aqueous pores in the lipid bilayers that close a few minutes after the electric field is withdrawn. The penetration of these pores is through the SC providing a pathway for the drug penetration via iontophoresis and/or electroosmosis [4, 42, 45, 53, 104].

3.4 Evaluation of transdermal Ketamine delivery

3.4.1 Medical needs for non - invasive Ketamine products

The need for transdermal Ketamine delivery was initiated from the clinicians' side. It is well known, that induction of anaesthesia by inhalation, injection, or intubation is a stormy procedure in children. In addition, painful and frightening experiences may cause long-term psychologic complications and make subsequent contacts with health professionals more difficult. As a result, a variety of premedications administered via various routes have been introduced, e.g. rectal administration of Ketamine [105]. It has been shown that rectally administered Ketamine alone produced dose-dependent sedative effects in children. Only a few studies reported about the antinociceptive potential of the transdermal Ketamine [106-108], but nobody has investigated the hypnotic effects of this drug after local administration. Quan et al., [107] have shown that topical Ketamine reduced pain in patients with no systemic side effects, indicating negligible or no generalized absorption. Azevedo et al., [108] have found that a controlled transdermal delivery of Ketamine prolonged the time to first rescue analgesic medication without adverse effects after minor gynecological surgery.

3.4.2 Evaluation of drug parameters

Ketamine is a well-known general anesthetic and short acting analgesic in use for almost 3 decades. The recent discovery of the N-methyl-d-aspartate (NMDA) receptor and its links to pain processing and spinal neural plasticity triggered renewed interest in Ketamine as a potential anti-hyperalgesic agent given its actions as a non-competitive NMDA-receptor antagonist. Ketamine has found a niche as a general anesthetic agent for various procedures in anesthesia and emergency medicine. Its undesirable psychic emergence effects and cardiovascular stimulating properties, however, have limited its usefulness. It therefore, remains a controversial drug to many anesthetists who remain wary of its adverse effects. However, it is becoming increasingly clear that a distinction must be made between the use of high-dose Ketamine as an anesthetic agent and the use of low-dose Ketamine for analgesic or anti-hyperalgesic effects [109].

Ketamine was first synthesized by Stevens [110] as a further development of phencyclidine and its congener cyclohexamine and was approved for clinical use in 1970.

The Ketamine molecule 2-(o-chlorophenyl)-2-(methylamino) cyclohexanone has a molecular weight of 238, is soluble in water to 20%, and has a pKa of 7.5. The aqueous solutions of Ketamine hydrochloride in clinical use have a pH range from 3.5 to 5.5. Under physiologic conditions the uncharged form of Ketamine is highly lipid soluble (10 times that of thiopentone. It is available as a racemic mixture that contains equal amounts of the two isomers S(1)-Ketamine and R(2)-Ketamine. In both animals and humans S(1)-Ketamine is 3±4 times more potent than R(2)-Ketamine for pain relief [111].

Low-dose Ketamine as the sole analgesic agent reduces pain significantly using the following routes: i.m., s.c. and i.v. However a number of qualifications apply: i.m. Ketamine may be suitable for short-term use only, and i.v. Ketamine appears to provide satisfactory pain relief only at the upper end of the low-dose range (1 mg/kg) with an increased risk of psychotomimetic adverse effects. For certain clinical conditions (e.g. asthma, allergies) taking the risk±benefit relationship into consideration, Ketamine (i.m., s.c., i.v.) might provide an alternative to conventional opioid analgesia. There is little evidence that epidurally administered low dose Ketamine by itself provides effective postoperative analgesia, which is in line with recent findings in a rat model for postoperative pain. Since Ketamine may be associated with spinal toxicity it is recommended that Ketamine not be injected intraspinally in humans.

There is a growing body of evidence that low-dose Ketamine may play an important role in postoperative pain management when used as an adjunct to local anesthetics, opioids, or other analgesic agents [112-116].

3.4.3 Possibilities for enhancement of Ketamine absorption through the skin

As Ketamine hydrochloride is a hydrophilic drug the possibility for enhancement is either by (1) hydration of the skin ex. Urea, (2) addition of penetration enhancer's ex. Surfactants, or (3) the use of special vehicles ex. organogel, hydrogel, liquid crystals. Some of these possibilities are used in the experimental part of this work.

4. EXPERIMENTAL

4.1 Materials

The active agent was Ketamine hydrochloride [Ketamine] a dissociative anesthetic (chemical name: (±)-2-(*o*-Chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride; molecular formula: $C_{13}H_{16}ClNO$. HCl; Molecular weight: 274.19). Ketamine was used in solution form (Calypsol®, Richter Ltd.). Another agent used was Ephedrine hydrochloride a sympathomimetic (chemical name: (-)-alpha-(1-methylaminoethyl)benzyl alcohol; molecular formula: $C_{10}H_{15}NO$. HCl; Molecular weight: 165.24) (Richter Ltd.).

The following materials were used as components for organogel, o/w cream, hydrogel, reference gel (electrode gel) and liquid crystal: liquid petrolatum, isopropyl myristate, amphiphilics: cetostearyl alcohol, Polysorbate 80, Brij 96V (poly-oxyethylene-10-oleyl ether) (ICI Hungary Ltd., Budapest), Carbopol 940, 971P (S&D Chemicals Ltd.) Miglyol 812 (fractionated coconut oil), Imwitor 900 (Dynamit Nobel Huls AG Witten, Germany), other additives: triethanolamine, carbamide, sodium hydroxide, glycerol (Hungaropharma Co., Budapest).

The materials for w/o/w (multiple) emulsions were as follow: 2,2,4,4,6,6,8-heptamethylnonane /Isohexadecane/ (Arlamol HD, Uniqema, Uniqema grade) and vegetable oil derivatives: avocado oil /Persea Gratissima/ (Symrise, Cosmetics grade), corn germ oil /Zea Mays/ (Naturol, Cosmetics grade), polyoxyethylene (30) dipolyhydroxystearate /PEG-30 Dipolyhydroxystearate/ (Arlacel P135, Uniqema, Uniqema grade), block copolymer of polyethylene oxide and polypropylene oxide /Poloxamer 407/ (Synperonic PE/F 127, Uniqema, Uniqema grade). carbomer (Carbopol Ultrez-10, BF. Goodrich, Ph.Eur.4th).

The polymer for the representative hydrogel systems for rheological evaluation was Hostacerin PN 73 (polyacrylate sodium-polyacrylamid copolymer) in concentrations between 1-3% (Hoechst).

All components used were of Ph. Eur. 4th grade.

4.1.1 Preparation of developed products

Hydro Gel

Carbopol 971 P	0.1 g.
Triethanolamine	0.2 g.
Carbamide	0.5 g.
Ketamine HCl	0.1 g.
Distilled Water	9.1 g.
	10.0 g. Total

Hydrogel type forms were formulated as follows: reference gel was a Carbopol 940 gel, neutralized by means of sodium hydroxide; in case of the hydrogel, Carbopol 971P was used and it was added first to distilled water. Then 1% carbamide was dissolved with 1% Ketamine HCl, followed by adding triethanolamine for neutralization.

Hostacerin PN 73	0.1 g.
Carbamide	0.5 g.
Ketamine HCl	0.1 g.
Distilled Water	9.3 g.
	10.0 g. Total

Hostacerin PN 73 was used and it was added first to distilled water. Then 1% carbamide was dissolved with 1% Ketamine HCl.

Organo Gel

Mineral Oil	7.7 g.
Invitor 900	1.3 g.
Distilled Water	0.9 g.
Ketamine HCl	0.1 g.
	10.0 g. Total

The organogel was prepared by heating a mixture of Miglyol 812 and Imwitor 900 to 70 °C. The melt was left to cool down at room temperature under continuous stirring, while the aqueous solution of 1% Ketamine was added drop wise.

To be an organo gel the water phase should be a maximum of 10% of the total preparation.

Liquid Crystals

Brij 96V	4.25 g.
Glycerine	1.75 g.
Liquid Paraffin	1.00 g.
Ketamine HCl	0.10 g.
Distilled Water	2.90 g.
	10.0 g. Total

The lyotropic liquid crystal samples were produced by heating the mixture of the liquid petrolatum, the glycerol and of Brij 96V to 80 °C. Distilled water and 1% Ketamine HCl was heated up to the same temperature and was added during constant stirring at 500 r/s (Ikamag RET-G magnetic stirrer). Stirring was continued until the mixture cooled down to room temperature. It takes about 3 days for proper crystal formation.

O/W Cream

Polysorbate 80	0.8 g.
Cetostearyl Alcohol	1.2 g.
Isopropyl Myristate	2.0 g.
Carbamidum	1.0 g.
Distilled Water	4.9 g.
Ketamine	0.1 g.
	10.0 g. Total

The preparation of the o/w cream was performed as follows: Polysorbate 80, cetostearyl alcohol and isopropyl myristate were melted together and mixed. The aqueous phase containing carbamide and 1% Ketamine HCl was then heated up to similar temperature. Finally the phases were mixed.

Multiple w/o/w emulsions were formulated with the two-step technology. The two-step technology was started as follows: the simple w_1/o emulsion was prepared by adding the w_1 aqueous phase to the oil phase containing the hydrophobic surfactant. Both phases were heated separately to 75 °C and then mixed. After the homogenization process (5 minutes at 1000 – 13500 rpm), the emulsion was cooled down to room temperature with gentle stirring. This w_1/o emulsion was dispersed – at a low stirring rate of 500 rpm (BIOMIX LE-402/LABORMIM, Hungary, DI 25 IKA-VERKE GmbH. Germany) – in the w_2 aqueous phase at room temperature. Ketamine was dissolved in the water of both phases.

4.2 Methods

4.2.1 Evaluation of rheological parameters of different dosage forms

A Haake Rheostress 1 rheometer (ThermoElectron, Germany) was used to measure the rheological properties of the creams. The flow curve test (increased shear rate at constant shear time and temeperature) and constant stress test were carried out as viscosity

measurements. A cone-plate (CP4/40 and 1/35° TI) combination was used as the measuring system. The temperature of the sample was 25 ± 0.1 °C. The tests were performed in triplicate. Relative standard deviation was 1.5-6.0%.

The cone-plate measuring device was also used for the oscillatory measurements of the systems. The cone angle was 1 degree, and the thickness of the sample was 0.048 mm in the middle of the cone. The measurements were performed at room temperature. The samples were kept in a space saturated with water vapour during measurement in order to prevent evaporation. The linear viscoelastic range was determined in the first step by examining the complex modulus as the function of shear stress at a given frequency (1 Hz). Based on these experiments, the value of shear stress was set at 2.5 Pa during the dynamic test as this value was always within the linear viscoelastic range, then the values of the storage and loss moduli were examined as the function of frequency.

4.2.2 In vitro drug release studies

Franz Diffusion Cell System (Hanson Research Co., USA) containing 6 cells, and equipped with autosampler (Hanson Microette Autosampling System) was used for the in vitro drug release and penetration measurements of the drug. The area for diffusion was 1,767 cm^2, and the receptor chamber volume was 7 ml. Cellulose acetate membranes (Machenerey-Nagel, Germany) with an average pore size of 0.45 μm were used. Pretreatment of the membrane was achieved by soaking in the receiving medium for the drug release studies. The receptor medium used was a phosphate buffer (pH= 5.4). The experiments ran at 32 ± 0.5 °C. 0.40 g samples of different compositions were placed evenly on the surface of the membrane, and 800 μl samples were taken after 0.5; 1; 2; 3; 4; 5; and 6 hours and replaced with fresh receptor medium. The absorbance was measured by a UV-spectrophotometer (Unicam UV-Vis Spectrofotometer) at a wavelength of 269 nm. in the case of Ketamine hydrochloride, and at 256 nm. in the case of Ephedrine hydrochloride. The blank vehicles without active agents served as references. The results of a 6 hours time period were plotted according to the diffusion model of Higuchi [117]. The results were expressed as the mean ± S.D. of four experiments.

4.2.3 In vitro penetration experiments

All the experimental conditions were similar to that of the in vitro drug release studies, except the fact that the synthetic membrane used was soaked in isopropyl myristate (IPM).

4.2.4 Evaluation of in vitro release and penetration data

The cumulative amount (Q) of drug released per surface area of membrane can be calculated as follows (Equation 4):

$$Q = \{ CnV + \sum_{i=1}^{n-1} Ci\, S \}/A \qquad (4)$$

Where

Q = Cumulative amount of drug released per surface area of membrane ($\mu g/cm^2$),

Cn = Concentration of drug ($\mu g/ml$) determined at the sampling interval,

V = Volume of individual Franz diffusion cell,

$\sum_{i=1}^{n-1} Ci$ = Sum of concentrations of drug ($\mu g/ml$) determined at sampling intervals 1 through n-1,

S = Volume of sampling aliquot, 0.2 ml,

A = Surface area of sample ($1.767 cm^2$).

4.2.5 In vivo studies of physiological changes after drug administration

After institutional approval had been obtained from the animal care committee, male Wistar rats weighing 240 ± 4.8 g were studied. The animals were kept on a 12 h light/12 dark cycle with food and water ad libitum. All experiments were carried out in the

same period of the day (1 to 4 p.m.) to exclude diurnal variations in pharmacologic effects. Each rat was tested only once. One day prior to the application of the cream, the back of each rat was carefully shaven and the skin was cleaned by wiping with water-containing cotton under seduxen-xylazin anesthesia. On the day of the experiment, the animals were anesthetized with the intraperitoneal injection of Seduxen® and xylazine (1mg/kg and 10 mg/kg intraperitoneal, respectively). Each cream (1.0 g) was applied onto the dorsal skin of the rat (about 15 cm^2/kg) 5 min after the injection, and covered with a protective overlay, an elastic adhesive bandage. The nonwoven PE cloth was fixed with Coban® adherent wrap. Control animals were exposed to the placebo cream (vehicle without active agent) in the same amount. The cream and the overlay were removed after the termination of the experiment.

Loss of the righting reflex was used to determine the presence of anesthesia, and its length in minutes was referred to as the duration of hypnosis. This technique is widely used for the assessment of anesthesia in rodents, and good agreement has been observed between the results with general anesthetics presented in various papers [118, 119]. Hypnosis was regarded as the state in which an animal could be placed on its back without righting itself. During the anesthesia the breathing was also determined in every 5 minutes (breathing frequency). 90 min after the Ketamine administration the animals were overdosed with Phenobarbital and 2ml blood sample was taken for HPLC study.

The rats were treated randomly according to one of the following protocols: the control group received the vehicle without Ketamine (n=10); the experimental animals were exposed to different vehicles (hydrogel, liquid crystal, o/w cream, organogel) (n=4-7) containing 1% w/w Ketamine. The data is presented as means ± S.E.M. The statistical analysis of the difference between different treatments was performed with the Student t test. A probability level less than 0.05 was considered as significance.

4.2.6 Evaluation of systemic absorption of Ketamine

Sample preparation involved extraction into diethyl ether and back extraction to 0.025 M sulphuric acid as reported by Adams et al. [120] The aqueous phase was dried and reconstituted in 0.01 M sodium dihydrogenphosphate and loaded onto the chromatographic column. Separation of Ketamine was performed on the method described by Gross et al. [121]. The HPLC (Knauer, Berlin, Germany) comprised a single pump isocratic system

connected to a flow-through UV detector measuring absorption at 215 nm. Chromatograms were collected and evaluated using Eurochrom 2000 software. The analytical separation was carried out on a reversed-phase Ultremex 5 μm CN column (250×4.6mm) (Phenomenex, Torrance, CA, USA). The mobile phase was methanol-acetonitril-ortophosporic acid-0.01 M sodium dihydrogenphosphate (200:80:2:718) adjusted to pH of 2.34. All chromatography was performed at ambient temperature, and the flow rate was 1 ml/min.

4.3 Results and discussion

4.3.1 Rheological characterization of dosage forms used

As there are well-established models to describe the effect of viscosity on the drug release from different semisolid systems, first of all a series of rheological measurements were preformed in order to evaluate the role of viscosity – in my case – on the drug release from hydrogels (the hydrogel dosage form was chosen because it had the highest release/penetration of all the dosage forms used). The drug release from hydrogels depends on the gel structure. This well known fact by now- was described by Higuchi [117] as all the mathematical models that originate from this era evaluating the liberation rate from the polymer matrix and the effect of the interaction between the matrix and the active agent. The general Higuchi model is as follow (Equation 5):

$$\frac{M_t}{A} = \sqrt{D\,(2c_0 - c_s)\,c_s\,t} \qquad (5)$$

Where

M_t = the amount of drug released at „t" time,

A = the surface,

D = the diffusion coefficient of the active agent,

c_0 = the concentration of the active agent at t=0,

c_s = the solubility of the active agent in the matrix.

The essence of the equation is, that the released drug amount (M) linearly increases with square root of time (t), the concentration, the solubility and the diffusion rate can be summed in a „*k*" emphirical constant (see Equation 6):

$$M_t = M_0 + kt^{0.5} \quad (6)$$

where M_0 is the drug release belonging to 0 time, therefore it's value is practically 0.

Erős and co-workers confirmed the applicability of this model several times [122 - 126]. They also estimated, that the model is independent from the chemical entity of the matrix; is also valid for hydrocarbon systems and for coherent emulsions and polymer matrices. The examples mentioned above referred to mainly for suspended active agents. I found it important to clarify whether or not the Higuchi mathematical model is valid also for dissolved active agents. The two active agents (Ketamine hydrochloride, Ephedrine hydrochloride) were examined in an aqueous polymer hydrogel.

The rheological behaviour of the matrices was studied paralelly with the estimation of the concentration dependency of the rheological parameters; the drug release kinetics, and the relationship between the viscosity and the release. Figure 1 shows the flow curves of different polymer containing hydrogels.

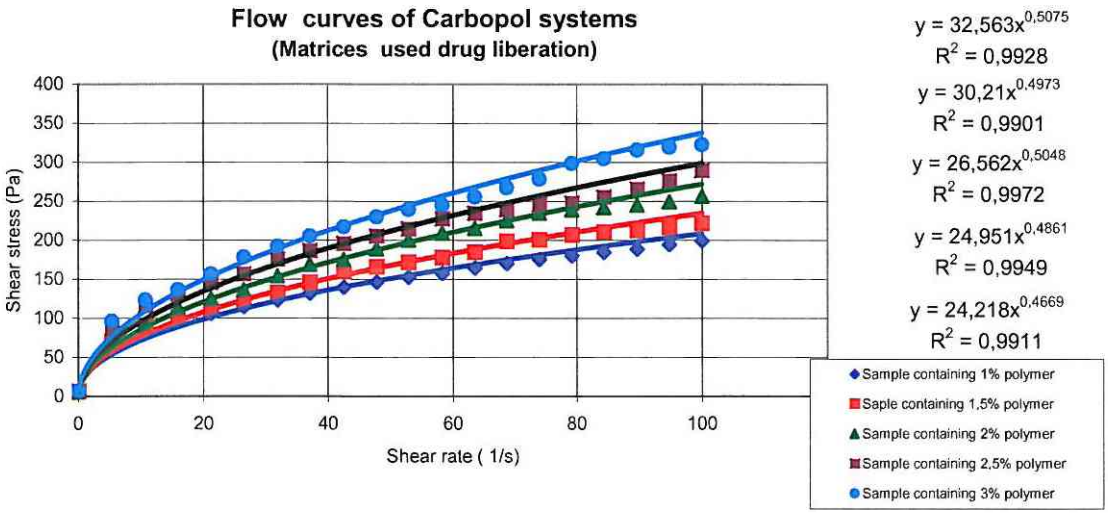

Fig. 1: Flow curves of hydrogels containing polymers in different concentrations.

The polymer content of Carbopol gels was between 1.0 - 3.0 % . In rheological terms, these systems were plastic gels with negligible yield value. Evaluation of the polymer concentration on viscosity can be found in Fig. 2.

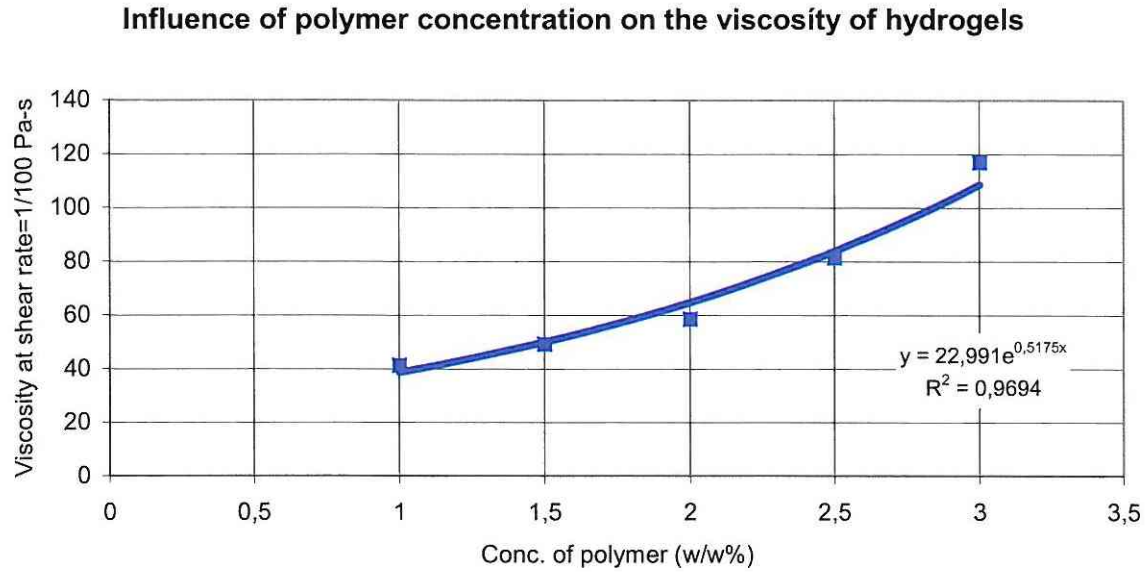

Fig. 2: Influence of the polymer concentration on the viscosity of hydrogels.

As seen in Fig. 2, the viscosity of the hydrogels increased exponentially with increasing polymer concentration. The next step was to evaluate the correlation of release (liberation) in the function of polymer concentration (Fig. 3).

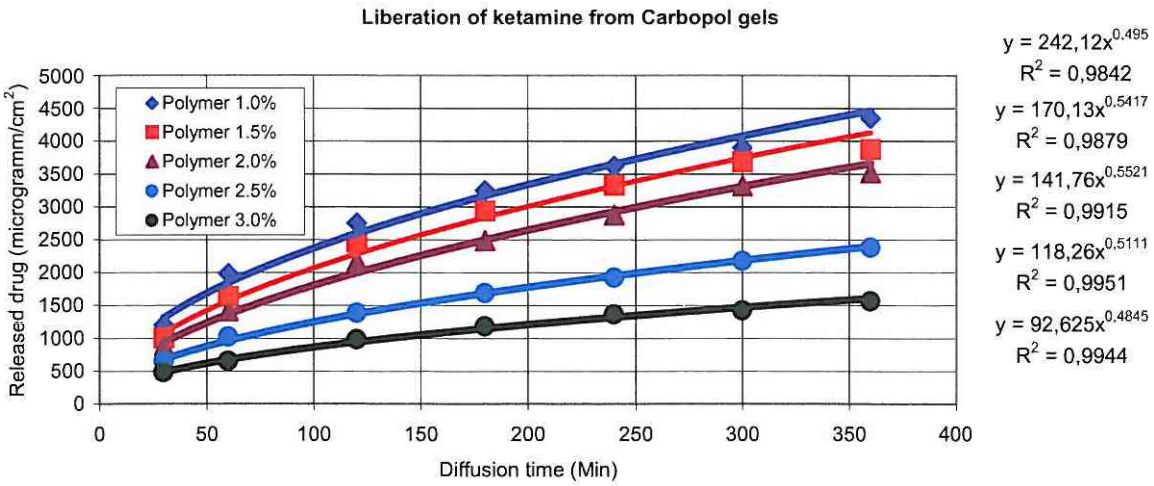

Fig. 3: Libaration of Ketamine from hydrogels.

Fig. 3 Illustrates the release kinetics. These kinetic curves – power functions, with a 0.5 exponent (see the equations on the figure). This fact justifies that the diffusion of dissolved molecules from a polymer matrix can be also described by the Higuchi equation.

The k values show the decrease tendency (242.12-92.63) with the increase of polymer concentration of Carbopol.

The following figure (Fig. 4) illustrates the relationship between the viscosity and the release.

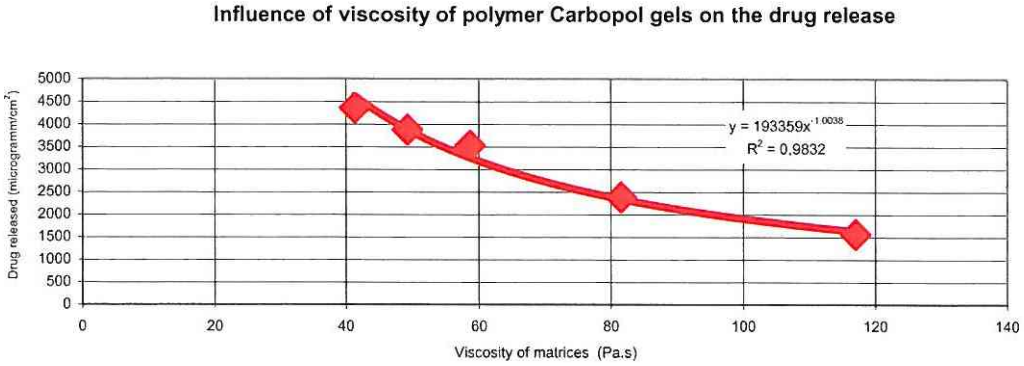

Fig. 4: Influence of viscosity of Carbopol gels on the release of Ketamine.

Fig. 4 Illustrates, that the viscosity of the gels greatly influences and determines the drug release process. The relationship between the viscosity and the drug release can be described by a non-linear regression equation (power-function). The correlation belonging to the curve-fitting is 0.983; which proves the good correspondence between the measured and the calculated data.

An another polymer, Hostacerin PN 73 (a polyacrylate salt and polyacrilamide) was also used for evaluation. The concentration of the gel forming polymer was within the range of 1.5-3.0%. The viscosity values of the matrices were similar to that of the ones previously studied (Fig. 5)

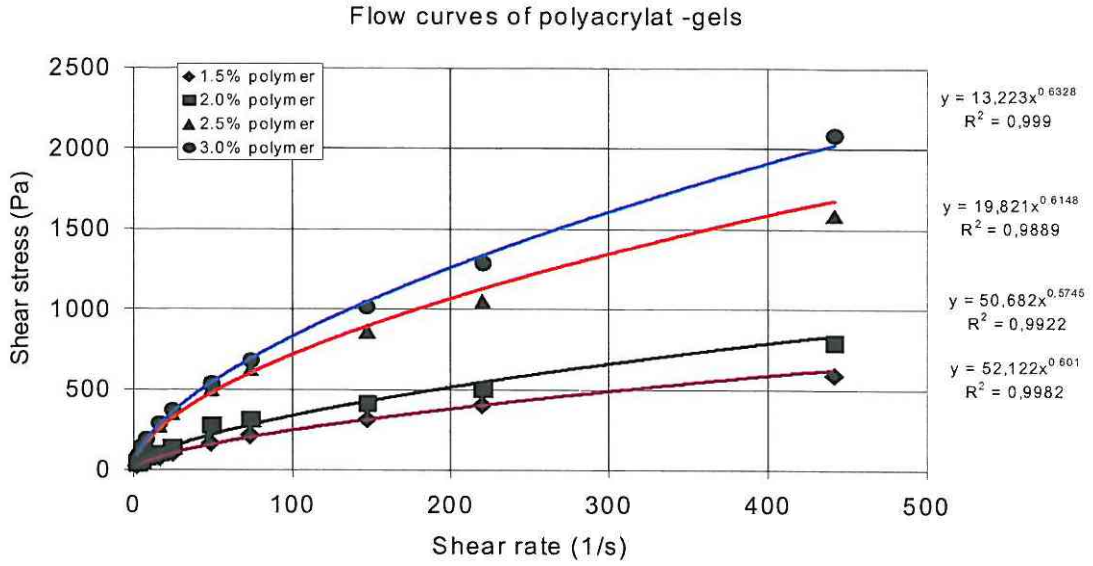

Fig. 5: Flow curves of polyacrylat Hostacerin gels.

These gels were plastic gels with negligible yield values as well. The resistance of the structure against shear (the flow curve) can be described by a power-function. The concentration dependecy of the viscosity can also be characterized with an exponential function (Fig. 6).

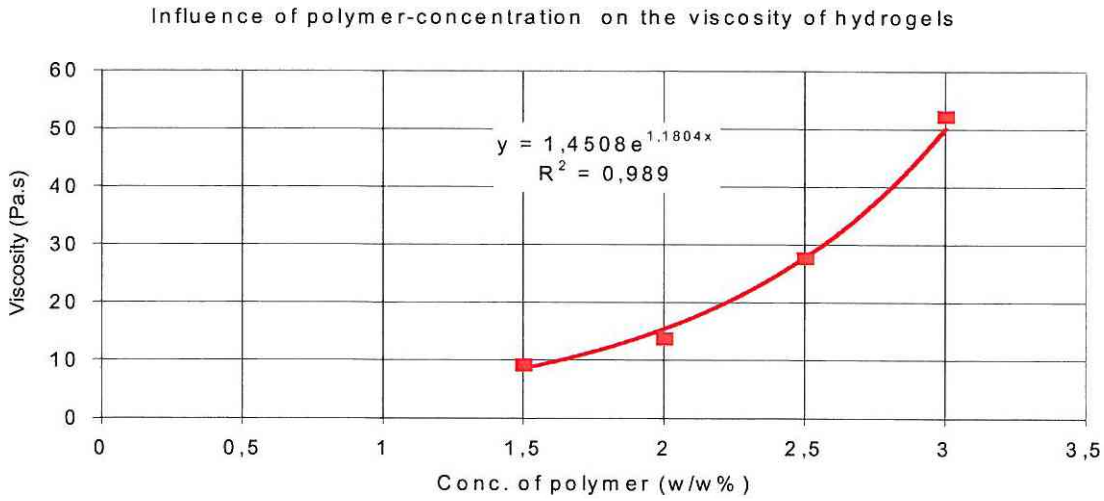

Fig. 6: Influence of polymer concentration on the viscosity.

The following figure (Fig. 7) shows the drug release process from these gels.

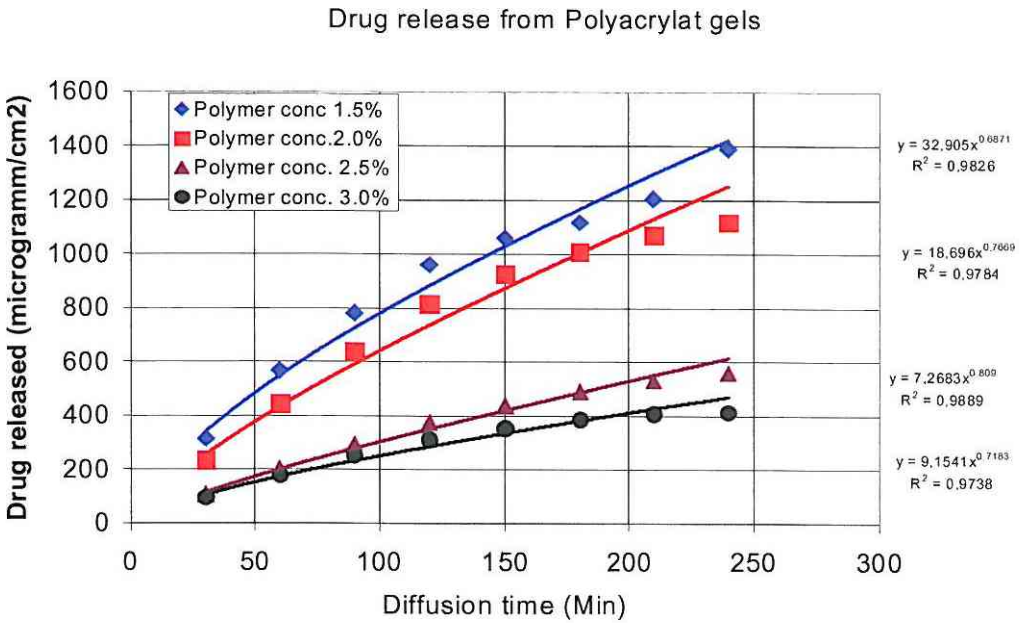

Fig. 7: Drug release from polyacrylate gels.

Functions expressing the drug release also power-function type, in general if the exponent is between 0.5 and 1 then we except the process to follow the Higuchi equation. The following figure (Fig. 8) evaluates the influence of viscosity on the released drug amount from the gels.

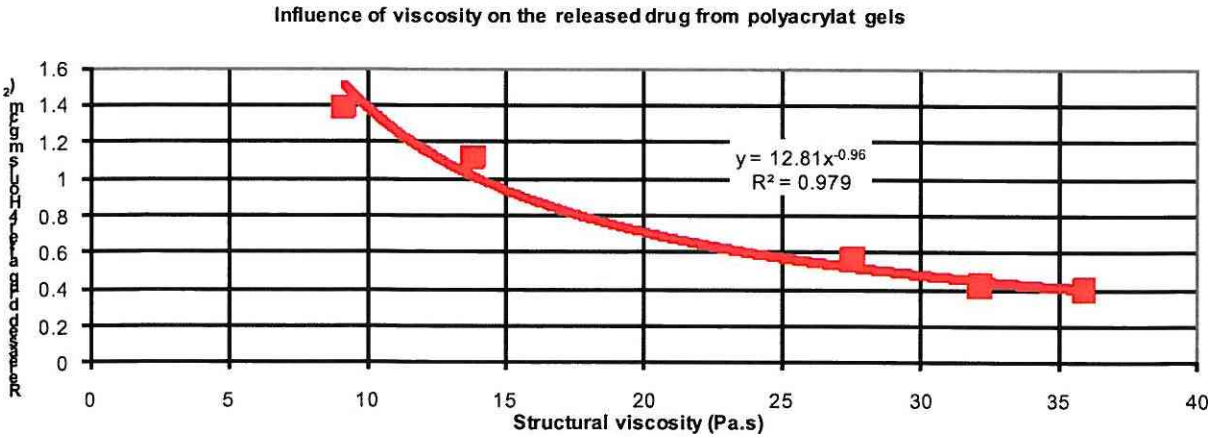

Fig. 8: Influence of viscosity on released drug amount from gels.

Fig. 8 Illustrates, that also in this case, the viscosity of the matrix is an important factor. The correlation coefficient of the exponential equation describing the process is 0.979, which gives a good verification of my hypothesis.

My work was expanded also on the evaluation of the constant representing the slope of the flow curves with the drug release rate. The constant of the flow curve reflects to the flexibility and the aligment tendency of the polymer chains. These chains are the barriers in front of the diffusing molecules, thus the rate of drug liberation is basicly dependent of this phenomena. Fig. 9 shows this comparison:

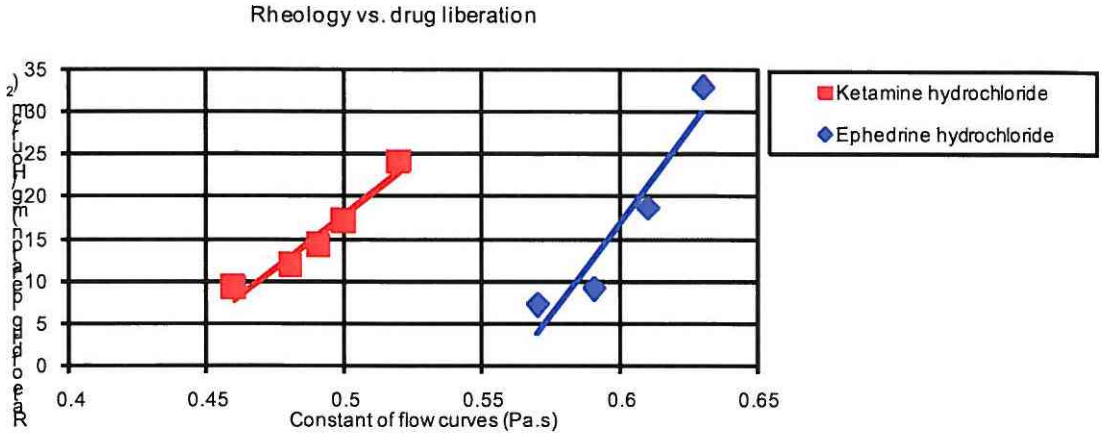

Fig. 9: Comparison of the flow curve constants with the drug liberation rate.

■ – Ketamine HCl in a Carbopol gel

♦ – Ephedrine HCl in a Hostacerin gel

The data in Fig. 9 verifies our theory. The more flexible the chains were, and the more tendency for aligment they had, the higher the liberation rate became.

4.3.2 In vitro drug release studies

The release process from different vehicles was measured through a synthetic membrane, which was soaked in the acceptor phase. Fig. 10 shows the cumulative release of the Ketamine amount plotted against square root of time ($\sqrt{t}$) for the determination of the release rate from the vehicles.

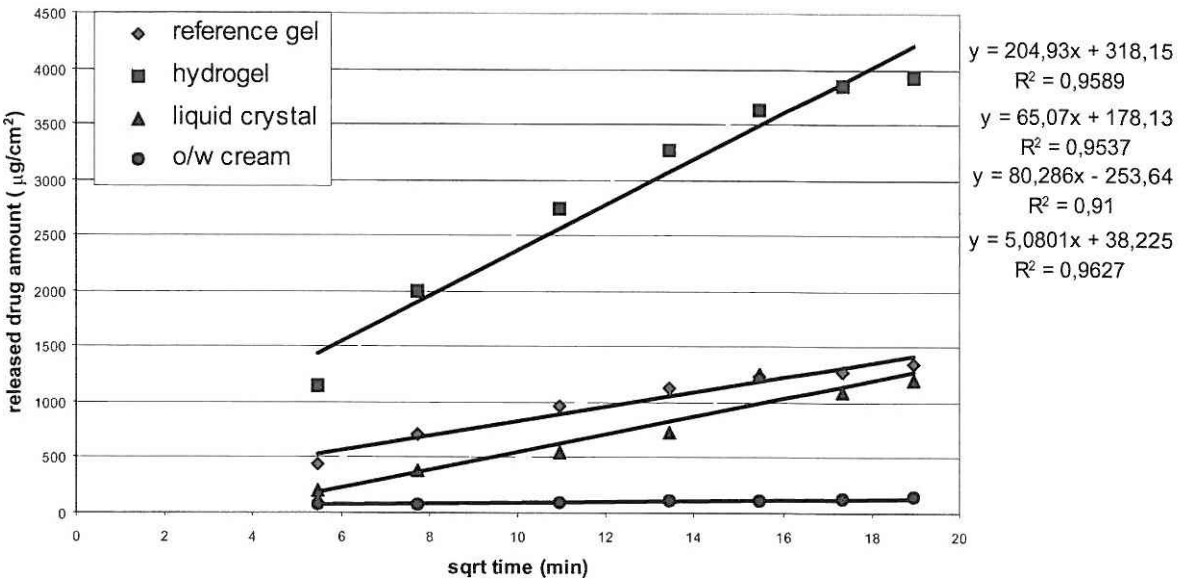

Fig. 10: Cumulative released Ketamine through a synthetic membrane soaked in buffer solution.

A hydrogel > reference gel > liquid crystal > o/w cream order was set based on the fluxes measured from the developed products. Differences between these rates are mainly due to the differences in the rheological parameters of the systems as both the dispersed state of the drug (dissolved), and its concentration were constant in this study.

Table 1 Summarizng the slopes representing the diffusion rates and also the released drug amounts after 2 hours, are compared when the penetration is through a hydrophilic environment.

Compositions	**In vitro released drug amount after half hour of treatment (µg/cm²)**	**In vitro released drug amount after 1 hour of treatment (µg/cm²)**	**In vitro released drug amount after 2 hour of treatment (µg/cm²)**
Reference gel	436.37	702.88	964.22
Hydrogel	1141.31	1998.20	2748.90
Liquid crystal	196.88	382.90	76.43
O/W cream	69.78	76.43	90.02

Table 1: Released Ketamine amounts after 0.5; 1; and 2 hours of treatment through buffer soaked membrane under in vitro conditions.

4.3.3 In vitro penetration experiments

When impregnating the membrane with IPM, the following changes were detected (Fig. 11), the parameters of the data presented here are listed in Table 2.

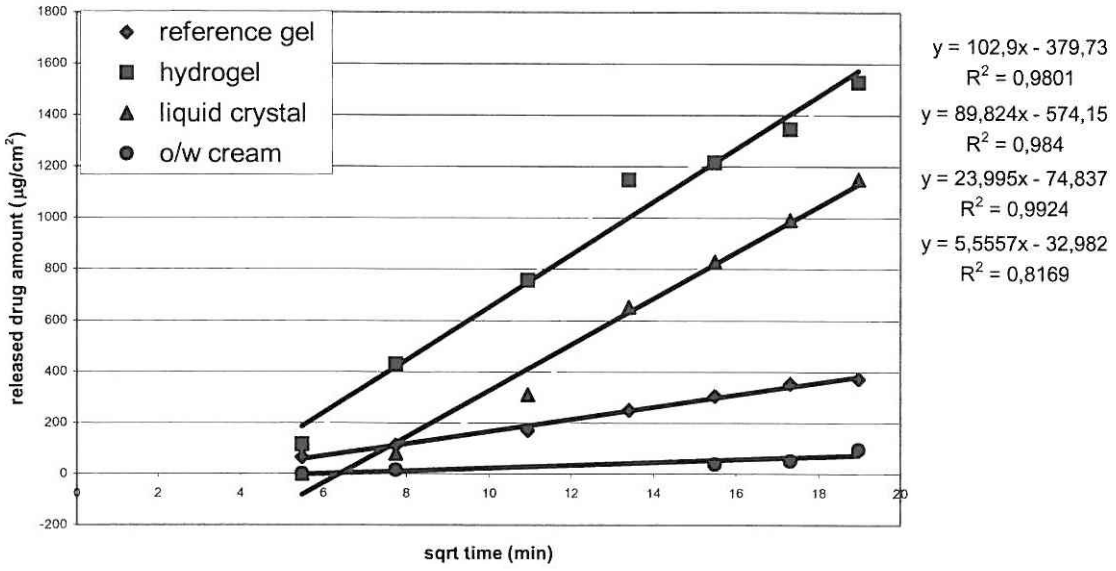

Figure 11: Cumulative released Ketamine through synthetic membrane soaked in IPM.

Table 2 Summarizes the slopes representing the diffusion rates and also the released drug amounts after 2 hours, are compared when the penetration is through a lipophilic environment.

Compositions	**In vitro released drug amount after half hour of treatment (µg/cm²)**	**In vitro released drug amount after 1 hour of treatment (µg/cm²)**	**In vitro released drug amount after 2 hour of treatment (µg/cm²)**
Reference gel	63.62	111.45	168.98
Hydrogel	114.04	429.94	756.14
Liquid crystal	0	79.83	309.54

O/W cream	0	13.81	25.4

Table 2: Released Ketamine amounts after 0.5; 1; and 2 hours of treatment through IPM soaked membrane under in vitro conditions

Reference gel and hydrogel fluxes decreased significantly when the membrane was soaked in IPM, while the liquid crystal system with high surfactant content did show a significant change. The presence of penetration enhancers in the developed products is well manifested, which is promising and shows ability to alter skin permeability properties as well. While the drug release through a synthetic membrane was mainly influenced by the rheological properties of the vehicles, diffusion ability through the IPM membrane is the consequence of enhancer present in the formulations.

I used an emulsion type dosage form as a vehicle in this experiment and I wanted to see if it would be better to use a simple or a multiple emulsion. As seen in figures 12 and 13 the multiple emulsion had a higher release rate compared to the simple emulsion.

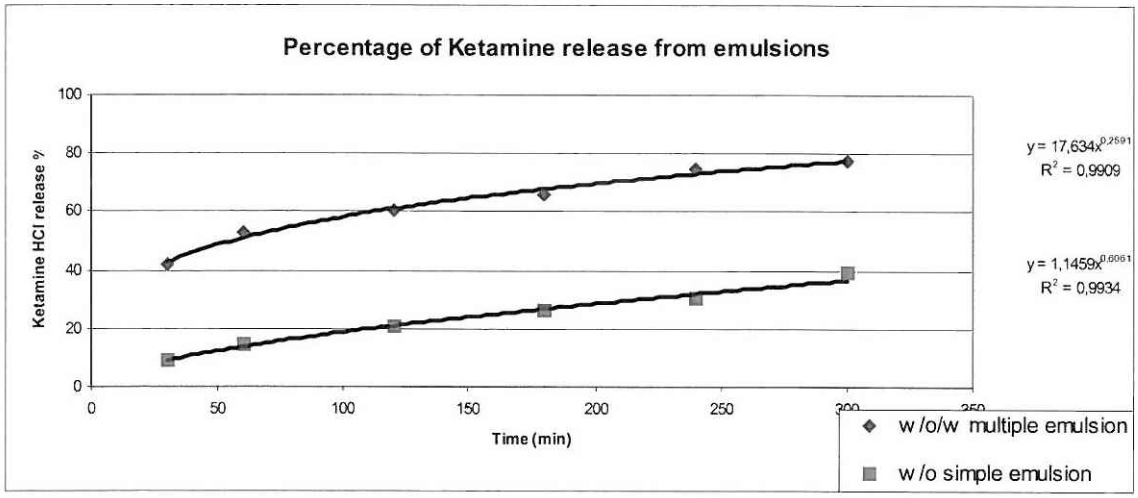

Figure 12: Percentage of Ketamine release from emulsions plotted against time.

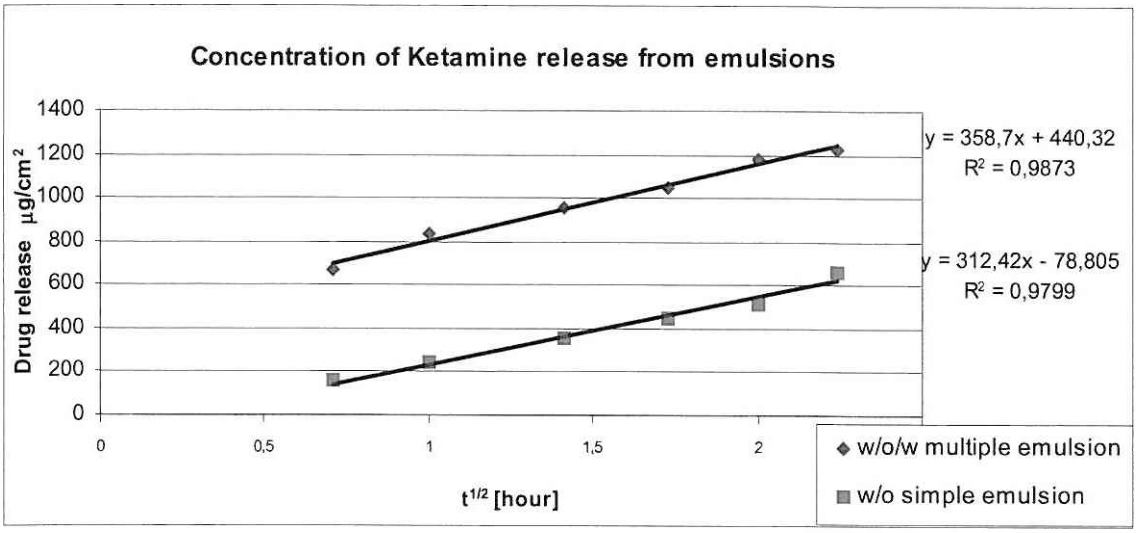

Figure 13: Concentration of Ketamine release from emulsions plotted against square root of time ($\sqrt{t}$).

At the end of the in vitro experiments I decided to use the hydrogel, organogel, liquid crystals, and a simple w/o emulsion as vehicles. I decided not to use the w/o/w multiple emulsion because it was found to be unstable as there was seperation of the components and as a result liquified.

We investigated the in vitro release and in vitro penetration of Ketamine vs. viscosity of matrices relationship, but there was not a significant correlation between biopharmaceutical data (eg. released drug amount, rate of release, rate of penetration) and the rheological data of matrices with different chemical composition.

4.3.4 In vivo studies of physiological changes after drug administration

Products containing 1% Ketamine, selected on the basis of the in vitro experiments were evaluated by different physiological tests. Fig. 14 shows the effect of Ketamine containing products on breathing frequency, asleeping time, and duration of sleep. The time needed for the appearance of the first urine was also detected.

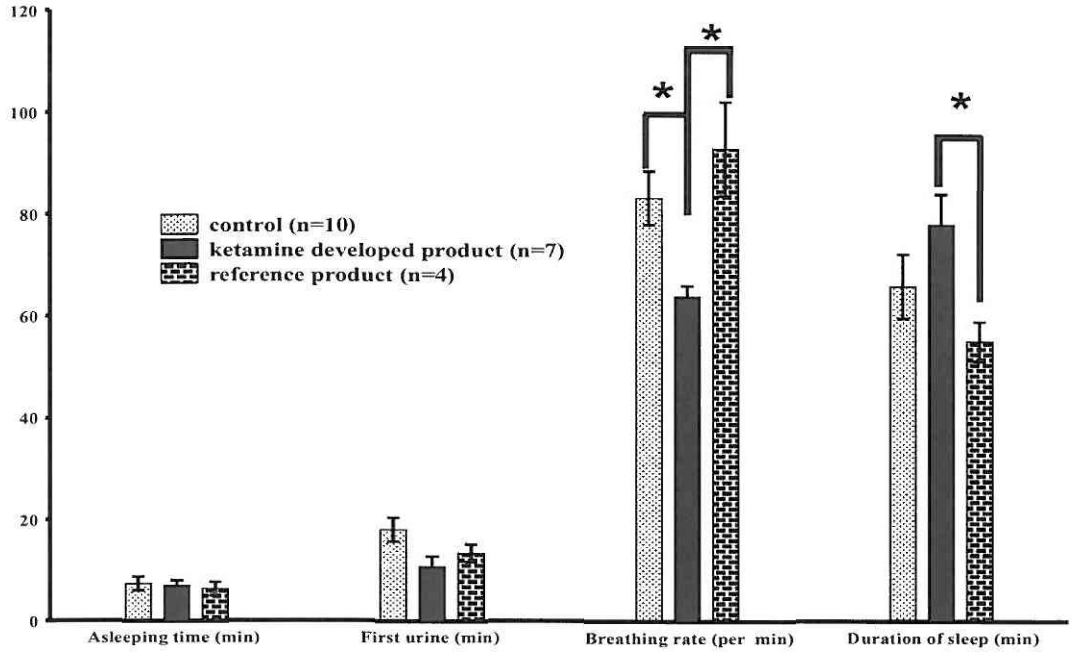

Figure 14: The in vivo effect of Ketamine on the different physiological parameters in anaesthetized rats. * = significant difference.

- Control – Electrode gel with Ketamine
- Ketamaine developed products – The vehicles with Ketamine
- Refrence product – The vehicles without ketamine

There were no significant differences between the groups in respect of the onset of hypnosis, however the duration of the hypnosis significantly increased in the Ketamine developed products compared to the other two groups. The breathing frequency significantly decreased in the Ketamine developed product group compared to the other two groups.

My results show that the developed products (hydrogel, liquid crystal and o/w cream) containing 1% Ketamine, have significant potency in in vivo circumstance, while the reference gel does not have any potentiating effect on the duration of the hypnosis nor significant influence in the breathing frequency.

4.3.5 Evaluation of systemic absorption of Ketamine

The plasma levels of Ketamine were determined 90 minutes after topical application of various formulations by HPLC with UV detection (Fig. 15, 16).

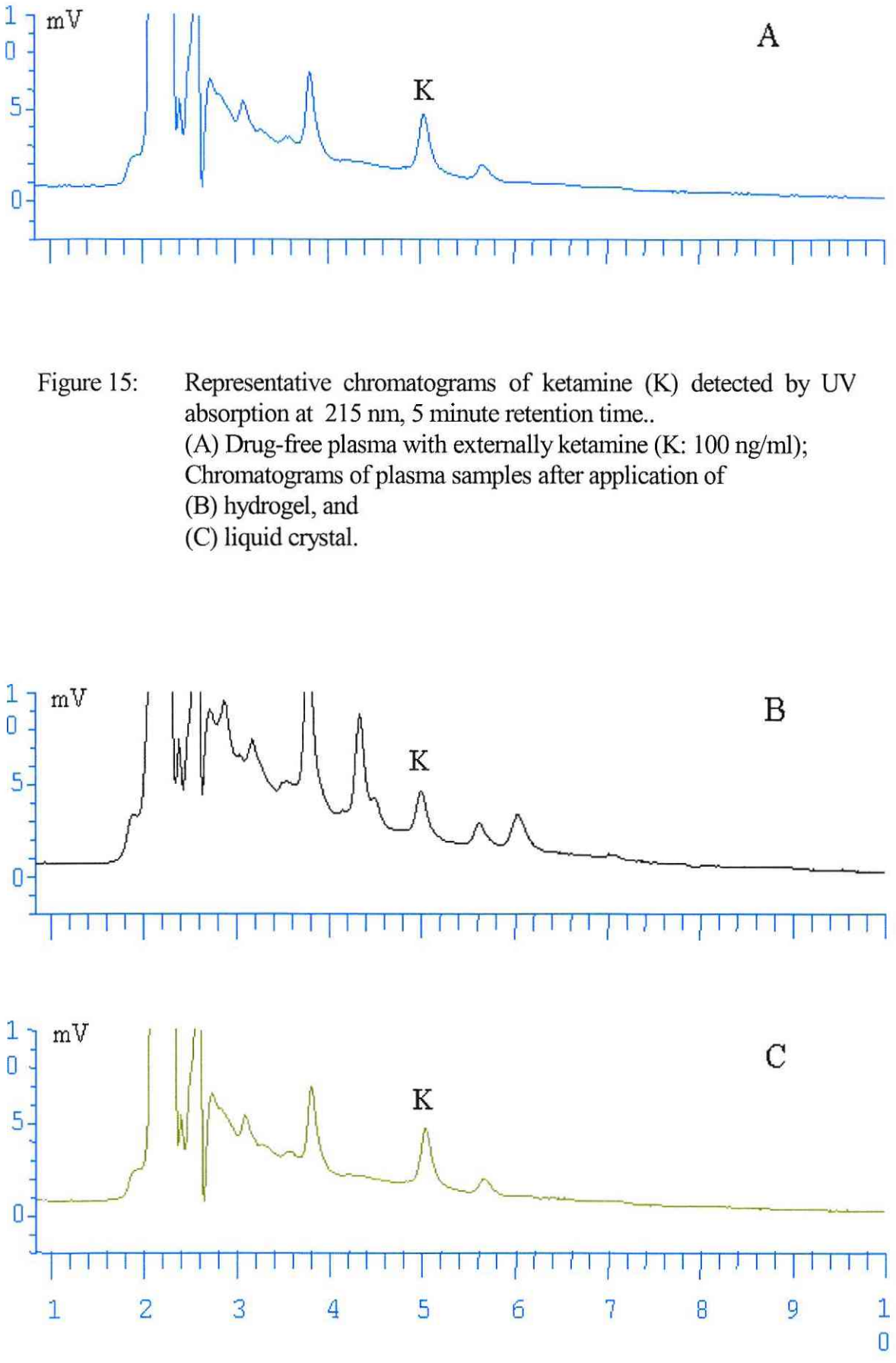

Figure 15: Representative chromatograms of ketamine (K) detected by UV absorption at 215 nm, 5 minute retention time..
(A) Drug-free plasma with externally ketamine (K: 100 ng/ml);
Chromatograms of plasma samples after application of
(B) hydrogel, and
(C) liquid crystal.

The retention time of Ketamine was 5.0 min. Measurable amount of Ketamine were detected, but there was no significant difference between the hydrogel and the liquid crystal products. Significant differences in the breathing rate and the duration of sleep were measured, this made it necessary to investigate the blood levels in time course. Fig. 17 shows the results of these experiments. After the application of the Ketamine containing preparations, the drug appeared in the blood, while it did not occur in the control group. The lowest level was 75 ng/ml while in some animals it reached 550 ng/ml (not shown in graph). The lowest level could be observed with o/w cream, while the highest level with the hydrogel, but the difference was not significant between the two groups.

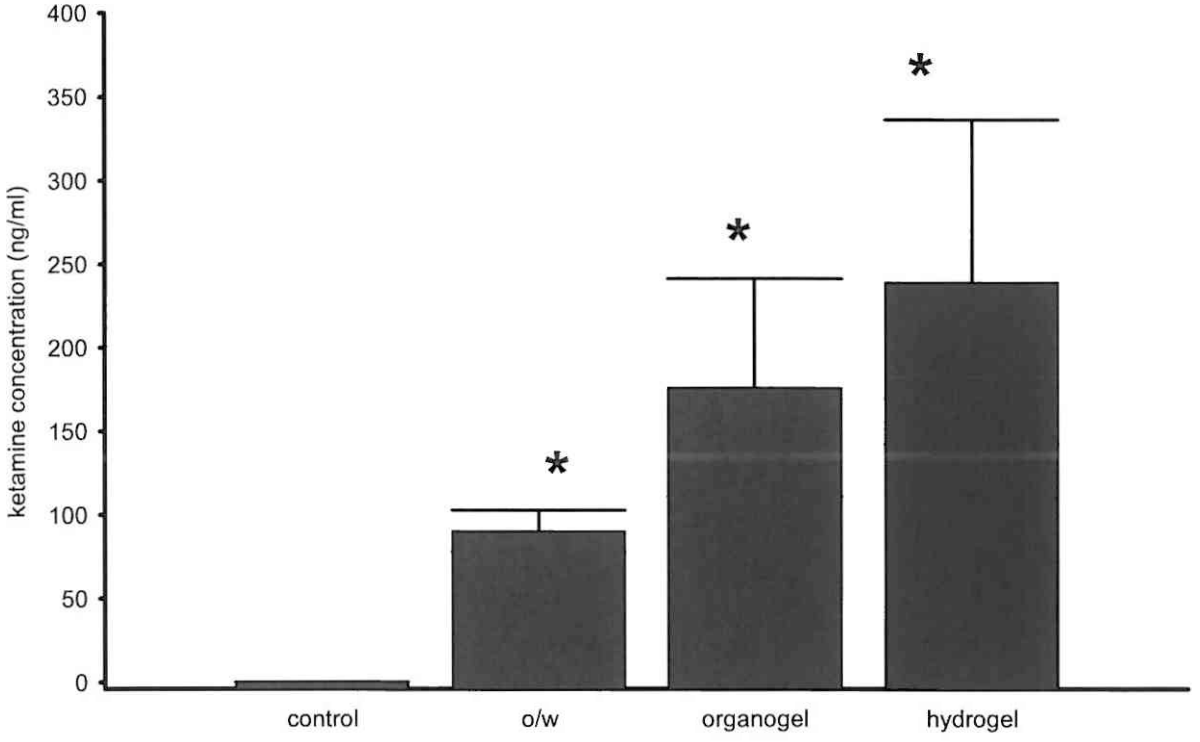

Fig. 16: Ketamine blood concentrations from different vehicles (*: significant differences from the control group), taken 90 minutes after topical application.

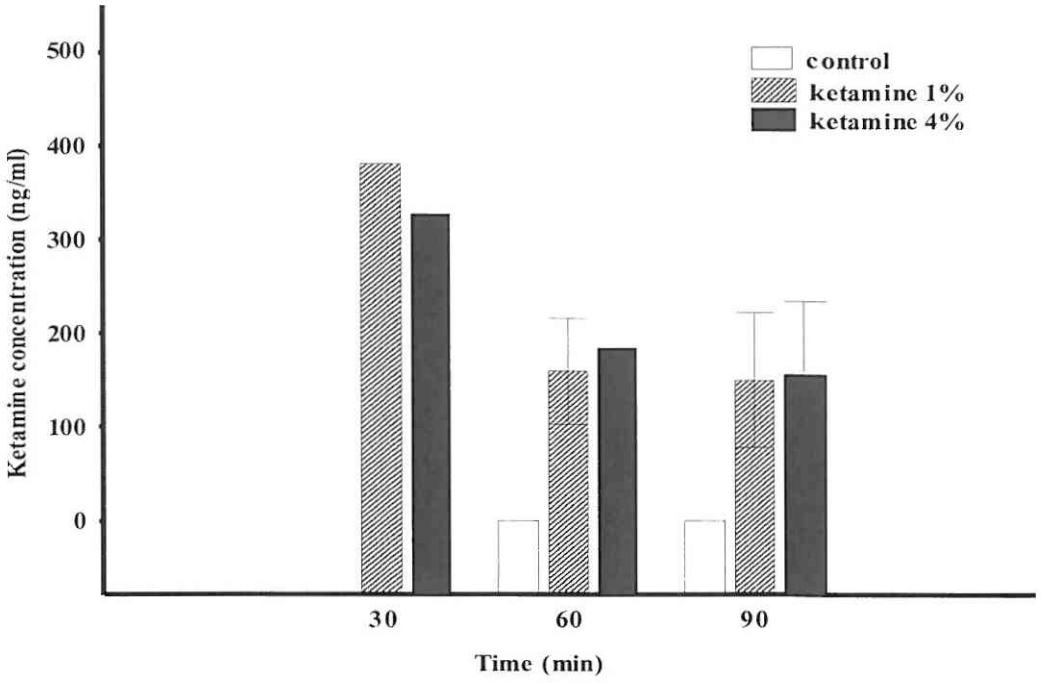

Fig. 17: Ketamine plasma levels from Hydrogel preparations containing 1% and 4% Ketamine taken 30, 60, and 90 minutes after topical administration.

My results show that Ketamine diffused through the skin and appeared in the blood. All the developed products might be good for this drug administration; however, the 1% Ketamine vehicle in this volume is a low dose. Even when I used 4% concentration there was negligible difference between the two.

It can also be concluded; that the in vitro evaluation method was more sensitive and the difference among the vehicles was overestimated in these cases. Further studies should be performed with higher drug concentrations for the characterization of the differences in the pharmacodynamics of the drug with different vehicles and to evaluate the correlation between the in vitro and in vivo absorption.

5. SUMMARY

A technological alternative for the induction of anasthesia has been long sought after by clinicians to reduce or eliminate the pain occured by this procedure. It is even more benificial in the situation of children. I have chosen Ketamine hydrochloride, a water soluble salt to be administered transdermally. The ideal method for administration would be a transdermal patch, which contains a semisolid dosage form. As a result I have chosen 4 vehicles: hydrogel, organogel, liquid crystals, and emulsion to be used as the vehicle. The vehicles were tested by the Frans Diffusion Cell System which contains 6 cells (2 cells containing the vehicle only and the other 4 had the vehicle with Ketamine). The experiment took 6 hours during which samples were taken at 0.5, 1, 2, 3, 4, 5, 6 hours. The first time the membrane was soaked in buffer solution to simulate hydrophilic conditions and the second the membrane was soaked in IPM to simulate lipophilic conditions. In both situations all the vehicles showed significant penetration through the membrane. It was high in the buffer soaked membrane due to the fact a hydrophilic drug was used, and it was lower in the IPM soaked membrane, but there still was significant penetration enhanced effects from the vehicles.

I chose hydrogel to compare the influence of viscosity on the release of the drug because it had the highest penetration rate. Two hydrogels were prepared, one with Carbopol and the other Hostacerin PN73 as polymers with concentrations from 1-3%. The Haake Rheostress 1 Rheometer was used to measure the rheological properties. It was shown as the polymer content increased the viscosity also incerased but the release of the drug decreased accordingly. It was also shown that there was a difference of the two polymers in regards to the release of the drug. The drug release was higher in the case where the chains were flexible and had more of a tendency for alignment.

Because of the positive results from the in vitro experiments in vivo experiments were preformed by applying the vehicles on Wistar rats and the sleeping time, first urine, breathing rate, and duration of sleep were observed. There were no significant differences between the groups in respect of the onset of hypnosis, however the duration of the hypnosis significantly increased in the Ketamine developed products compared to the reference product. The breathing frequency significantly decreased in the Ketamine developed product group compared to the reference product. After 90 minutes of the vehicle application blood was taken to measure the concentration of Ketamine in the

plasma by the use of HPLC with UV detection. The result showed me there was penetration of Ketamine through the skin into the blood. Another series of tests was preformed where blood was taken at 30, 60, and 90 minutes after application of Ketamine with two different concentrations (1% and 4%).

In conclusion the use of vehicles showed a promising effect on the transdermal delivery of Ketamine in both in vitro and in vivo situations.

The aim of developing Ketamine containing semisolid dosage forms from which the drug reaches the systemic circulation was achieved, however further studies should be preformed with higher drug concentrations to evaluate the pharmacodynamic correlation between the in vitro and in vivo permeation.

6. REFERENCES

1. M. Foldvari, Non-invasive administration of drugs through the skin: challenges in delivery system design, PSTT Vol. 3, No. 12, December (2000) 417–425

2. C.S. Asbill and B.B. Michniak, Percutaneous penetration enhancers: local versus transdermal activity, PSTT 3 (2000) 36–41

3. B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur. J. Pharm. Sci. 14 (2001) 101–114

4. B.W. Barry, Drug delivery routes in skin: a novel approach, Adv. Drug Del. Rev. 54 Suppl. 1, (2002) 31–40

5. W. Barry, Is transdermal drug delivery research still important today?, DDT 6 (2001) 967

6. R. Neubert and W. Wohlrab, In vitro methods for the biopharmaceutical evaluation of topical formulations, Acta. Pharm. Technol. 36 (1990) 197–206

7. J.D. Segers and J.L. Zatz, Techniques for Measuring In Vitro Release From Semisolids, Dissolution Technol. 5 (1998) 3–13

8. W.A. Hanson, State of the Art in Dissolution Testing of Transdermal Dosage Forms. Pham. Sci. Group Meeting, Scarborough, Ontario, December (1989)

9. K.T.E. Kierstan, A.E. Beezer, J.C. Mitchell, J. Hadgraft, S.L. Raghavan and A.F. Davis, UV-spectrophotometry study of membrane transport processes with a novel diffusion cell, Int. J. Pharm. 229 (2001) 87–94

10. S.A. Wissing and R.H. Müller, Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration, J. Contr. Rel. 81 (2002) 225–235

11. N.K. Yogeshvar and R.H. Guy, Modeling transdermal drug release, Adv. Drug Del. Rev. 48 (2001) 159–172

12. L.A. Bunge, Release rates from topical formulations containing drugs in suspension, J. Contr. Rel. 52 (1998) 141–148

13. J. Hadgraft, M.E. Lane, Skin permeation: The years of enlightenment, Int. J. Pharm. 305 (2005) 2–12

14. K.A. Walters, M.S. Roberts, The Structure and Function of Skin, Chapter 1, pp.1–40 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York, (2002), Edited by: K.A. Walters

15. T.K. Ghosh, A.K. Banga, Methods of Enhancement of Transdermal Drug Delivery: Part 1, Physical and biochemical Approaches, Pharm. Tech., March (1993) 72–98

16. M. Wang, F.C. Lin, L.Y. Chen, J.Y. Lai, Application of asymmetric TPX membranes to transdermal delivery of nitroglycerin, J. Contr. Rel. 50 (1998) 187–195

17. I.J. Bosman , K.Ensing, R.A. de Zeeuw, Selection and evaluation of anticholinergics for transdermal drug delivery, Int. J. Pharm. 169 (1998) 75–82

18. J.J. Collins, MBBS, FRACP, I.J. Dunkel MD, S.K. Gupta PhD, C.E. Inturrisi PhD, J. Lapin RN, L.N. Palmer RN, BSN, S.M. Weinstein MD, R.K. Portenoy MD, Transdermal fentanyl in children with cancer pain: Feasibility, tolerability, and pharmacokinetic correlates, J. Ped. March (1999)

19. S.Davarana, M.R. Rashidib, R.Khandaghic, M.Hashemid, Development of a novel prolonged-release nicotine transdermal patch, Pharm. Res. 51 (2005) 233–237

20. R.Jewelewicz M.D., New developments in topical estrogen therapy Fertility and Sterility, Vol. 67, No. 1. January (1997) 1–12

21. J.C. Stevenson, Optimising delivery systems for HRT, Maturitas 33 (1999) 31–38

22. F.Wu.U. Srinivas-Shankar, Testosterone replacement therapy, Medicine, Volume 33, Issue 11, 1 November (2005) 41–43

23. D.Giugliano, R.Acampora, R.Marfella, C. La Marca, M. Marfella, F. Nappo, F. D'Onofrio, Hemodynamic and Metabolic Effects of Transdermal Clonidine in Patients With Hypertension and Non–Insulin-Dependent Diabetes Mellitus, Am. J. Hypertension, Volume 11, Issue 2, February (1998) 184–189

24. P.J. Lee, N. Ahmada, R. Langer, S. Mitragotri, V. Prasad Shastri, Evaluation of chemical enhancers in the transdermal delivery of lidocaine, Int. J.Pharm. 308 (2006) 33–39

25. C. Sansom, Transdermal patch formulation offers breakthrough in ADHD treatment, Pharm. Sci. & Tech. Today, Volume 2, Issue 8, 1 August (1999) 307–308

26. W. E. Pelham, L. Burrows-MacLean, E. M. Gnagy, G. A. Fabiano, E. K. Coles, K. E. Tresco, A. Chacko, B. T. Wymbs, A. L. Wienke, K. S. Walker and M. T. Hoffman, Transdermal Methylphenidate, Behavioral, and Combined Treatment for Children With ADHD, Experimental and Clinical Psychopharmacology, Volume 13, Issue 2, May (2005) 111–126

27. Y. Bayarski, Transdermal Drug Delivery, http://searchwarp.com/swa29948.htm, downloaded on March 7 2006

28. M.S. Roberts, S.E. Cross, M.A. Pellett, Skin Transport, Chapter 4, pp.89–197 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York (2002), Edited by: K.A. Walters

29. C. Ramachandran, D. Fleisher, Transdermal delivery of drugs for the treatment of bone disease, Adv. Drug Del. Reviews, Vol. 42 Iss. 3 (2000) 197–223

30. B. J. Thomas and B. C. Finnin, The transdermal revolution, Drug Discovery Today, Volume 9, August (2004) 697–703

31. S. Paparella, Transdermal Patches: An Unseen Risk For Harm, Journal of Emergency Nursing 31 (2005) 278–281

32. R. Pal, Rheology of double emulsions, Journal of Colloid and Interface Science 307 (2007) 509–515

33. R. Pal, Viscosity models for multiple emulsions, Food Hydrocolloids, Article in Press (2007)

34. R. Pal, On the viscoelastic behavior of multiple emulsions, Journal of Colloid and Interface Science, Article in Press (2007)

35. Tschierske, Amphotropic liquid crystals, Current Opinion in Colloid & Interface Science 7 (2002) 355–370

36. Editorial overview, Lyotropic liquid crystals, Current Opinion in Colloid and Interface Science 8 (2004) 424–425

37. C.L. Lester, C.A. Guymon, Ordering effects on the photopolymerization of a lyotropic liquid crystal, Polymer 43 (2002) 3707–3715

38. M. Sutter, J. Siepmann, W.E. Hennink, W. Jiskoot, Recombinant gelatin hydrogels for the sustained release of proteins, J. Cont. Rel. 119 (2007) 301–312

39. T. Coviello, P. Matricardi, C. Marianecci, F. Alhaique, Polysaccharide hydrogels for modified release formulations, J. Cont. Rel. 119 (2007) 5–24

40. S. Murdan, G. Gregoriadis, A. T. Florence, Inverse toroidal vesicles: precursors of tubules in sorbitan monostearate organogels , International Journal of Pharmaceutics, Volume 183, Issue 1, 10 June (1999) 47-49

41. K.A. Walters, K.R. Brain, Dermatological Formulation and Transdermal Systems, Chapter 7, pp.319–400 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York (2002), (Edited by: K.A. Walters)

42. B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur. J. Pharm. Sci. 14 (2001) 101–114

43. G. Cevc, G. Blume, A. Schatzlein, D. Gebauer, A. Paul, The Skin: a pathway for systemic treatment with patches and lipid-based carriers, Adv. Drug Del. Rev. 18 (1996) 349–378

44. J. Hadgraft, Skin, the final frontier, Int. J. Pharm. 224 (2001) 1–18

45. J.A. Bouwstra, P.L. Honeywell-Nguyen, G.S. Gooris, M. Ponec, Structure of the skin barrier and its modulation by vesicular formulations, Progress in Lipid Research 42 (2003) 1–36

46. F. Yamashita, M. Hashida, Mechanistic and empirical modeling of skin permeation of drugs, Adv. Drug Del. Reviews 55 (2003) 1185–1199

47. G.K. Menon, New insights into skin structure: scratching the surface, Adv. Drug Del. Reviews 54 Suppl. 1 (2002) 3–17

48. H.J. Dean, D. Chen, Epidermal powder immunization against influenza, Vaccine, Vol. 23, Issue 5, 16 December (2004) 681–686

49. C. Sarber, A.F. Davis, Bioavailability and Bioequivalence of Dermatological Formulations, Chapter 8, pp.401–498 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York (2002), (Edited by: K.A. Walters)

50. V.H. Lee, L. Narawane, Absorption Barriers, Chapter 1 pp.1–66 in Drug Absorption Enhancement, Harwood Academic Publishers, Chur (1994), (Edited: A. (Bert) G. de Boer)

51. P. Buck, Skin barrier function: effect of age, race and inflammatory disease, International Journal of Aromatherapy, Volume 14, Issue 2 (2004) 70–76

52. A.C. Watkinson, K.R. Brain, Basic Mathematical Principles in Skin Permeation, Chapter 3, pp.61–88 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York (2002), (Edited by: K.A. Walters)

53. J. Hadgraft, K.A. Walters, Skin Penetration Enhancement, Chapter 6 pp.177–198 in Drug Absorption Enhancement, Harwood Academic Publishers, Chur (1994), (Edited: A. (Bert) G. de Boer)

54. K. Moser, K. Kriwet, A. Naik, Y.N. Kalia, R.H.Guy, Passive penetration enhancement and it's quantification in vitro, European Journal of Pharmaceutics and Biopharmaceutics, 52 (2001) 103–112

55. A.B.J. Noach, M.A. Hurni, A.G. de Boer, and D.D. Breimer, The Paracellular Approach: Drug Transport and It's Enhancement via the Paracellular Parhway, Chapter 10 pp.291–404 in Drug Absorption Enhancement, Harwood Academic Publishers, Chur (1994), (Edited: A. (Bert) G. de Boer)

56. C.M. Lehr, The Transcytosis Approach, Chapter 11 p.325–366 in Drug Absorption Enhancement, Harwood Academic Publishers, Chur (1994), (Edited: A. (Bert) G. de Boer)

57. A.F. Davis, R.J. Gyurik, J. Hadgraft, M.A. Pellett, K.A. Walters, Formulation Strategies for Modulating Skin Permeation, Chapter 6, pp.271–318 in Drugs and Pharmaceutical Sciencies Volume 119, Marcel Dekker, Inc., New York, (2002), (Edited by: K.A. Walters)

58. V. M. Meidan, M. C. Bonner, B. B. Michniak, Transfollicular drug delivery - Is it a reality?, International Journal of Pharmaceutics 306 (2005) 1–14

59. G.W. Watson,, Wells, R.P.K., Willock, D.J. and Hutchings, G.J., A comparison of the adsorption and diffusion of hydrogen on the {1 1 1} surfaces Ni, Pd and Pt from density functional theory calculations. J. Phys. Chem. B 105 (2001) 4889–4894

60. R. Rurali, Hernandez, E., Godignon, P., Rebollo, J. and Ordejon, P., First principles studies of neutral vacancies diffusion in SiC. Comp. Mat. Sci. 27 (2003) 36–42

61. Y.C. Martin, 1978. Quantitative Drug Design., Marcel Dekker, New York.

62. G.P. Moss, Dearden, J.C., Patel, H. and Cronin, M.T.D., Quantitative structure–permeability relationships (QSPRs) for percutaneous absorption. Toxicol. in vitro 16 (2002) 229–317.

63. B.E. Vecchia, and Bunge, A.L., (2002). Evaluating the transdermal permeability of chemicals. In: Hadgraft, J. and Guy, R.H., Editors, (2002). Transdermal Drug Delivery (second ed.), Marcel Dekker Chapter 2, pp.22–55.

64. G.L. Flynn (1990). Physicochemical determinants of skin absorption. In: Gerrity, T.R. and Henry, C.J., Editors (1990). Principles of Route to Route Extrapolation for Risk Assessment, Elsevier, New York

65. R.O. Potts and Guy, R.H., Predicting skin permeability. Pharm. Res. 9 (1992) 663–669

66. D. Fitzpatrick, J. Corish and B. Hayes, Modelling skin permeability in risk assessment––the future, Chemosphere 55 (2004) 1309–1314

67. Y.N. Kalia, R.H. Guy, Modeling transdermal drug release, Advanced drug delivery reviews, Vol. 48 Iss. 2-3 (2001) 159–172

68. P. Morganti, E. Ruocco, R. Wolf and V. Ruocco, Percutaneous absorption and delivery systems, Clinics in Dermatology, Volume 19, July-August (2001) 489–501

69. A. Naik, Y. N. Kalia and R. H. Guy, Transdermal drug delivery: overcoming the skin's barrier function, Pharm. Sci. & Tech. Today, Volume 3, Issue 9, 1 September (2000) 318–326

70. W.R. Pfister, D.S.T. Hsieh, Permeation Enhancers Compatable with Transdermal Drug Delivery Systems: Part I: Selection and formulation considerations, Pharm. Tech., September (1990) 132–140

71. Stowic Resourses Limited, Pharmalicensing, Zero Order Delivery Transdermal Technology, http://www.stowic.com, downloaded on Tuesday 18 December 2001

72. B.Y. Kim, H.J. Doh, T.N. Le, W.J. Cho, C.S. Yong, H.G. Choi, J. S. Kim, C.H. Lee, D.D. Kim, Ketorolac amide prodrugs for transdermal delivery International Journal of Pharmaceutics 293 (2005) 193–202

73. P. Suppasansatorn, G. Wang, B R. Conway, W. Wang, Y. Wang, Skin delivery potency and antitumor activities of temozolomide ester prodrugs, Cancer Letters Article in press (2006) 1–11

74. K. Moser, K. Kriwet, C. Froehlich, Y.N. Kalia, R.H. Guy, Supersaturation: Enhancement of Skin Penetration and Permeation of a Lipophilic Drug, Pharmaceutical Research, Vol.18 No.7 (2001) 1006–1011

75. J. Hadgraft, Skin deep, Eur. J. of Pharm. and Bio. Pharm 58 (2004) 291–299

76. A.C. Williams, B.W. Barry, Penetration enhancers, Advanced Drug Delivery Reviews 56 (2004) 603–618

77. M.H. Schmid, H.C. Korting, Liposomes as Penetration Enhancers and Controlled Release Units, Chapter 12.1, pp.323–334 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

78. P. N. Gupta, V. Mishra, A. Rawat, P. Dubey, S. Mahor, S. Jain, D.P. Chatterji and S. P. Vyas, Non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study, Int J. Pharm., Volume 293, April (2005) 73–82

79. E. Touitou, N. Dayan, L. Bergelson, B. Godin and M. Eliaz, Ethosomes — novel vesicular carriers for enhanced delivery: characterization and skin penetration properties, J.Cont. Rel., Volume 65, April (2000) 403–418

80. R.C. Wester, H.I. Mailbach, Penetration Enhancement by Skin Hydration, Chapter 2.1, pp.21–28 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

81. C. S. Asbill and B. B. Michniak, Percutaneous penetration enhancers: local versus transdermal activity, Pharmaceutical Science & Technology Today, Volume 3, Issue 1, 1 January (2000) 36–41

82. S.C. Chattaraj, R.B. Walker, Penetration Enhancer Classification, Chapter 1.2, pp.5–20 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

83. A.C. Williams, Urea and its Derivatives as Penetration Enhancers, Chapter 10.1, pp.289–296 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

84. K.A. Walters, Penetration Enhancers and their use in Transdermal Therapeutic Systems (1989), Chapter 10 pp.197–232 in Transdermal Drug Delivery, Marcel Dekker, Inc., New York (1989), Edited by J. Hadgraft, R.H. Guy,

85. D. A. Godwin, M. R. Player, J. W. Sowell, B. B. Michniak, Synthesis and investigation of urea compounds as transdermal penetration enhancers, Int. J. Pharm., 167 (1998) 165–175

86. B.B. Michniak, Amines and Amides as Penetration Enhancers, Chapter 4.4, pp.79–80 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

87. M. Hashida, F. Yamashita, Terpines as Penetration Enhancers Chapter 11.1, pp.309–322 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

88. B. Berner, P Liu, Alcohols, Chapter 3.1, pp.45–60 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

89. B. Bendas, R. Neubert, W. Wohlrab, Propylene Glycol Chapter 3.2, pp.61–78 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

90. S.B. Ruddy, Surfactants, Chapter 8.1, pp.245–258 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

91. M. R. Prausnitz, Microneedles for transdermal drug delivery, Advanced Drug Delivery Reviews, Volume 56, Issue 5 (2004) 581–587

92. M. Cormier, B. Johnson, M. Ameri, K. Nyam, L. Libiran, D. D. Zhang and P. Daddona Transdermal delivery of desmopressin using a coated microneedle array patch system, Journal of Controlled Release, Volume 97, Issue 3 (2004) 503–511

93. A. L. Teo, C. Shearwood, K. C. Ng, J. Lu and S. Moochhala, Transdermal microneedles for drug delivery applications, Materials Science and Engineering: B, In Press, Corrected Proof, Available online 22 March (2006)

94. Y. Xie, B. Xu and Y. Gao. Controlled transdermal delivery of model drug compounds by MEMS microneedle array, Nanomedicine: Nanotechnology, Biology and Medicine, Volume 1 (2005) 184–190

95. J. H. Park, M. G. Allen and M. R. Prausnitz, Biodegradable polymer microneedles: Fabrication, mechanics and transdermal drug delivery, Journal of Controlled Release, Volume 104, May (2005) 51–66

96. E. Camel, Ultrasound, Chapter 15.2, pp.369–382 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

97. I. Lavon and J. Kost, Ultrasound and transdermal drug delivery, Drug Discovery Today, Volume 9, Issue 15, August (2004) 670–676

98. S. Mitragotri and J. Kost, Low-frequency sonophoresis: A review, Advanced Drug Delivery Reviews, Volume 56, March (2004) 589–601

99. A. Joshi and J. Raje, Sonicated transdermal drug transport, Journal of Controlled Release, Volume 83, September (2002) 13–22

100. L. Machet and A. Boucaud, Phonophoresis: efficiency, mechanisms and skin tolerance, International Journal of Pharmaceutics, Volume 243, August (2002) 1–15

101. W.H.M. Craane-Van Hinsberg, J.C. Verhoef, H.E. Bodde, Transdermal Iontophoresis of Peptides And Proteins: Transport Kinetics and Electrical Skin Properties, Chapter 7 pp.199–220 in Drug Absorption Enhancement, Harwood Academic Publishers, Chur (1994), Edited: A. (Bert) G. de Boer

102. B.H. Sage, Jr, Iontophoresis, Chapter 15.1, pp.351–368 in Perercutaneous Penetration Enhancers, CRC Press, Inc., Florida (1995), Edited by: E.W. Smith, H.I. Maibach

103. Y. Wang, R. Thakur, Q. Fan and B. Michniak, Transdermal iontophoresis: combination strategies to improve transdermal iontophoretic drug delivery, Eur. J. Pharm. and Bio., Volume 60, July (2005) 179–191

104. A.R. Denet, R. Vanbever and V. Preat, Skin electroporation for transdermal and topical delivery, Adv. Drug Del. Rev., Vol. 56, March (2004) 659–674

105. M. Tanaka, M. Sat, A. Sait and T. Nishikawa, Reevaluation of rectal ketamine premedication in children, Anesthesiology 93 (2000) 1217–1224

106. K. Crowley, J.A. Flores and C.N. Hughes, Clinical application of ketamin ointment in the treatment of sympathetically mediated pain, Int. J. Pharm. Comp. 2 (1998) 122–127

107. D. Quan, M. Wellish and D.H. Gilden, Topical ketamine treatment of postherpetic neuralgia, Neurology 60 (2003) 1391–1392

108. V.M.S. Azevedo, G.R. Lauretti, N.L. Pereira and M.P. Reis, Transdermal ketamine as an adjuvant for postoperative analgesia after abdominal gynecological surgery using lidocaine epidural blockade, Anesth. Analg. 91 (2000) 1479–1482

109. http://www.erowid.org/chemicals/ketamine/ketamine_basics.shtml downloaded on June 4, 2007

110. Stevens (1963) http://www.ketamine.com/, downloaded on July 1 2007

111. S.Budavari, et al., Ketamine, The Merck Index An Encyclopedia of Chemicals, Drugs, and Biologicals (1996)

112. N. Elia, M.R. Tramèr, Ketamine and postoperative pain – a quantitative systematic review of randomised trials, Pain, Volume 113, Issues 1-2, January (2005) .61–70

113. C. Aveline, H. Le Hetet, P. Vautier, J.F. Gautier, F. Bonnet, Peroperative ketamine and morphine for postoperative pain control after lumbar disk surgery, Eur. J. Pain, Volume 10, Issue 7, October (2006) 653–658

114. M.F. De Kock, P.M. Lavand'homme, The clinical role of NMDA receptor antagonists for the treatment of postoperative pain, Best Practice & Research Clinical Anaesthesiology, Volume 21, Issue 1, March (2007) 85–98

115. O.N. Aydın, B. Ugur, S. Özgun, H. Eyigör, Ö. Copcu, Pain prevention with intraoperative ketamine in outpatient children undergoing tonsillectomy or tonsillectomy and adenotomy, J. Clin. Anesth., Volume 19, Issue 2, March (2007) 115–119

116. X. Wang, H. Xie, G. Wang, Improved postoperative analgesia with coadministration of preoperative epidural ketamine and midazolam, Journal of Clinical Anesthesia, Volume 18, Issue 8, December (2006) 563–569

117. T. Higuchi, Pharm. Sci. 50 (1961) 874–875

118. K.W. Miller, W.D.M. Paton, E.B. Smkith, R.A. Smith, Physicochemical approaches to the mode of action of general anaesthetics, Anesth. 36 (1972) 339–351.

119. G. Horvath, M. Szikszay, G. Benedek, Calcium channels are involved in the hypnotic anesthetic action of dexmedetomidine in rats. Anesth. Analg. 74 (1992) 884–888.

120. H.A. Adams, B. Weber, M.B. Bachmann, M. Guerin, G. Hempelmann, Anaesthesist 41 (1992) 619

121. A.S. Gross, A. Nicolay, A. Eschalier, Simultaneous analysis of ketamine and bupivacaine in plasma by high-performance liquid chromatography Journal of Chromatography B, 728 (1999) 107–115

122. I. Erős, T. Takács-Wormsdorff, Drug liberation from polymer-hydrogels. Part 1: Kinetics of the process, Pharmazie 52 (1997) 311–313

123. E.Y. Abu-Eida, I. Erős, Z. Aigner, K. Pintye-Hódi, M. Kata, Controlled drug release from coherent systems. Part 1: Liberation from hydrocarbon gels, Pharmazie 54 (1999) 786–787

124. I. Erős, E.Y. Abu-Eida, I. Csóka, Zs. Sánta, A. Cserne, T. Kövér, Optimization of drug release from dermatological semisolid preparation. Drug Dev. Research 59 (2003) 316–325

125. I. Erős, I. Csóka, E. Csányi, T. Takács-Wormsdorff, Examination of drug release from hydrogels, Polym. Adv. Technol. 14 (2003) 847–853

126. T. Pénzes, G. Blazsó, Z. Aigner, G. Falkay, I. Erős, Topical absorption of piroxicam from organogels - in vitro and in vivo correlations. Int. J. Pharm. 298 (2005) 47–54

Acknowledgements

I would like to thank

Prof. Dr. Habil. István Erős, Ph.D., D.Sc.

for allowing the opportunity to do my Ph.D. studies in the

Department of Phramaceutical Technology

I would like to thank

Prof. Dr. Habil. Piroska Révész , Ph.D., D.Sc.

for guiding in my last stages of my dissertation

I would like to thank

Dr. habil. Ildikó Csóka Ph.D.

for the many times she helped me the past years of my studies

and in the completion of this work